Monoclonal Antibodies in Cancer

Clinical trials of monoclonal antibodies in cancer therapy are beginning, but the best bet for their use may be in cancer detection

In the 8 years since their discovery, monoclonal antibodies have proved to be valuable research tools. Now, investigators are attempting to take the next step—to move the antibodies from the research laboratory into the clinic. Their first widespread use is likely to be for detecting cancer and following the response of patients to treatment. "In the next 5 years," predicts John Minna of the National Cancer Institute (NCI), "you will see a revolution in immune diagnosis that will clearly outweigh other things."

The application of monoclonal antibodies to general cancer therapy may be more difficult to achieve. A few small clinical trials have been reported and have shown limited success, but they have also pointed up the problems that will have to be solved if monoclonal antibodies are ever to be the long-sought "magic bullet" for cancer therapy.

In making monoclonal antibodies it is possible to produce virtually unlimited quantities of an antibody that reacts specifically with a particular antigen. The advantage of a monoclonal antibody, says Jeffrey Schlom of NCI, "is its reproducibility. For the first time, you can develop a reagent that you can use forever."

For cancer detection or therapy, what is needed are monoclonal antibody reagents that will react with antigens present on tumor cells but not on normal ones. Such antibodies, by homing in on their targets, might specifically kill the tumor cells by triggering an immune attack on them or might deliver cell-killing agents, such as chemotherapeutic drugs, radioactive isotopes, or toxins. Linked to a radioactive tracer, they could be used to locate primary or metastatic tumors in patients known or thought to have cancer. And, in an early warning system for detecting new cancers or cancer recurrences, they might serve as immunologic reagents for screening blood samples for the presence of tumor antigens shed into the bloodstream.

Within the past 2 or 3 years investigators in several laboratories have produced monoclonal antibodies that react with antigens on one or another of a number of different tumors, including the big three cancers in the United States: lung, colon, and breast. Others have been made against the antigens of melanomas, pancreatic cancer, and several kinds of lymphomas and leukemias. The list will certainly continue to grow.

The ideal of an antibody that reacts only with an antigen found in a single type of tumor may not be attainable, however, because there may be no such thing as a tumor-specific antigen. As Zenon Steplewski of the Wistar Institute told the First Annual Congress for Hybridoma Research, which was held in Los Angeles in February, "Up to now, there is not a single antigen that you could call tumor-specific—found on tumor cells but nowhere else. If you look long enough you will find it somewhere else." These days researchers speak of the "tumor-associated" antigen, which marks a particular tumor cell but may also be found on other types of tumor cells, on fetal cells, or on cells of normal tissue. The last of these possibilities constitutes the biggest obstacle to the therapeutic or diagnostic application of a monoclonal antibody directed against the antigen.

Nevertheless, researchers are turning up monoclonal antibodies with specificities that may be good enough for clinical use. Minna, Frank Cuttitta, and Steven Rosen of NCI screened 15,000 to 20,000 monoclonal antibodies in their search for one that would be specific for small cell carcinoma of the lung, which accounts for about 25 percent of the 130,000 lung cancers that occur annually in the United States.

To make monoclonal antibodies, researchers must first make hybridomas—hybrids of antibody-producing and myeloma cells. The NCI workers obtained the antibody-producing cells from the spleens of mice that had been immunized with small cells. After the antibody-producing cells were fused with mouse myeloma cells, clones were grown from individual hybrids. The antibodies produced by about 20,000 of the clones were screened against two different lines of cultured small cells and one line of noncancerous lymphoid cells. Eighty antibodies passed this initial screen, reacting with the cancer cells but not with the normal line.

Three of these have undergone extensive further testing, against both cancer cells and normal cells. One line reacted only with normal kidney cells and with breast cancer, neuroblastoma, and lung adenocarcinoma cells. The other two have reacted with nothing else so far, although they have not been tested against any kind of fetal cells. According to Minna, "you can use these antibodies to detect tumor in the presence of normal tissue." Some of the other monoclonal antibodies have proved less specific, reacting either with normal cells or with lines of tumor cells in addition to the small cell lines.

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Eventually it may be possible to make diagnostic use of monoclonal antibodies that are directed against small cell and other forms of lung cancer. Screening sputum samples from smokers, who are at high risk of developing lung cancer, is one such potential application.

Steplewski, Hilary Koprowski, and their colleagues at Wistar have made a monoclonal antibody to a line of colorectal cells that may prove useful for detecting cancers of the gastrointestinal tract. Victor Ginsburg and John Magnani of the National Institute of Arthritis, Metabolism, and Digestive Diseases have identified the colorectal cell antigen that reacts with the antibody as a glycolipid, a large monosialoganglioside. The glycolipid is not found on normal adult cells but is present on fetal cells.

Koprowski says, "This antigen is shed into the blood by cancer patients. We can use it for diagnosis." By using the monoclonal antibody, the Wistar group

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detected the antigen in blood from 23 of 33 patients with advanced colorectal cancers but not in blood from 38 healthy volunteers or from six patients with noncancerous bowel diseases. They also found the antigen in blood from patients with gastric and pancreatic cancers.

Similarly, researchers in the laboratory of Richard Metzgar at Duke University Medical Center have prepared a series of five monoclonal antibodies to pancreatic cancer cells and have begun characterizing the tumor antigens with which they react. One of the antigens is present in serum from patients with pancreatic cancer or any of several other adenocarcinomas.

Its blood concentration may have prognostic value. Six of seven patients with progressive pancreatic cancer had high concentrations of the antigen, whereas two of three patients with stable disease had low concentrations. Moreover, because pancreatic cancer is usually not diagnosed until it is too advanced to be treated, a simple method for early detection would be highly desirable. Metzgar hopes that the monoclonal antibodies will eventually permit the development of such a method.

Using monoclonal antibodies to screen blood samples for tumor antigens is one approach to cancer detection. Another combines the specificity of the antibodies with the techniques of nuclear medicine or of computer assisted tomography (CAT) to locate cancerous lesions in the patient's body.

At the Hybridoma Congress, Jean-Pierre Mach of the Ludwig Institute for Cancer Research described attempts* to locate colorectal cancers with radiolabeled monoclonal antibodies to carcinoembryonic antigen (CEA), a fetal antigen which is also made by these tumors. In one series of experiments, the patients were injected with antibody labeled with ^{131}I and images showing the distribution of radioactivity were made by a method analogous to that by which x-ray images are built by CAT scans. Areas of high radioactivity could be seen at the sites of the tumors but the spots were also present in additional locations, indicating lack of specificity.

More recently, Mach and his colleagues used radiolabeled preparations of the monoclonal antibody developed by the Wistar group in the same way. They detected the tumors in 40 percent of patients with colorectal cancer in one study and in 80 percent in another. Although Mach thinks the imaging methods are promising he does not think they are ready yet for clinical application.

If monoclonal antibodies can carry radioactive tracers to tumors for the purpose of detection, they may also be able to deliver cell-killing agents—radioisotopes, drugs, or toxins. Ricin, a toxin from castor bean plants, has been mentioned prominently as an agent that could be linked to monoclonal antibodies for the purpose of killing tumor cells. Keith Krolick, Jonathan Uhr, and Ellen Vitetta of the University of Texas Southwestern Medical School in Dallas recently showed that a conjugate of a ricin chain linked to a polyclonal antibody preparation could kill almost all the tumor cells in bone marrow from leukemic mice without destroying the stem cells that produce normal red and white blood cells.

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Stanley Order of Johns Hopkins Medical School has some promising results in treating inoperable liver cancers with polyclonal antibodies linked to a radioisotope. CAT scans show that the tumors may shrink more with this therapy than after treatment with standard drugs or external radiation. Order is exploring the similar use of monoclonal antibodies, but so far they have proved unsatisfactory because they lose the radioactive material shortly after being injected into the bloodstream.

Antibodies by themselves may lead to cell killing by triggering an immune attack on their targets. Investigators, including Ronald Levy and Richard Miller of Stanford University School of Medicine, Stuart Schlossman and Jerome Ritz of Sidney Farber Cancer Center and Harvard Medical School, and Ivor Royston of the University of California at San Diego have already used appropriate monoclonal antibodies to treat small numbers of lymphoma and leukemia patients. The patients generally experienced transient improvements in their symptoms, principally decreased numbers of tumor cells in their blood or other tissues, but in most cases the tumor cell counts went back up again about as fast as they came down.

The most dramatic results so far were obtained with a patient treated for a recurrent B cell lymphoma by the Stanford group.† In lymphomas of this type, all the tumor cells are members of a clone and they carry on their surfaces the same antibody chain. The Stanford workers made a monoclonal antibody directed specifically against the antibody of the patient's tumor cells. Treatment with the monoclonal antibody produced a remission of the disease that has lasted some 8 months since the therapy was stopped. Because this approach requires tailoring of the monoclonal antibody to the individual lymphoma patient, it would present practical difficulties if additional work bears out the initial success.

Although the clinical trials suggest that monoclonal antibodies can lead to tumor cell death, they also point up the many problems that will have to be solved before their promise for therapeutic application is realized. One significant factor that may limit therapeutic applications of monoclonal antibodies is the presence of "blocking factors"—tumor antigens that bind with the antibody—in the blood of some patients. These factors can prevent binding of the antibodies to tumor cells, an effect the Sidney Farber investigators observed in one patient. This effect may be less in patients with smaller tumor burdens.

In addition, the binding of some antibodies to cells causes the target antigen to disappear from the cell surface. This antigenic modulation, which was seen in two studies with leukemic patients, may decrease the effectiveness of repeat doses of antibody. Not all antibodies have this effect, however, and those that do may be identifiable by testing them against the target cells beforehand.

Another potential problem is related to the fact that at present there are no monoclonal antibodies of human origin that can be used for cancer therapy or detection. The monoclonal trials have been done with material of mouse origin, and Order obtained his polyclonal antibodies from a number of different animals. Investigators are concerned that patients will suffer a severe allergic reaction (anaphylaxis) in response to the repeated infusion of a foreign protein,

*Performed in collaboration with investigators at the University of Lausanne, the University of Geneva, and the Institut Gustave Roussy and the Institut Radiobiologie Clinique, which are both in Villejuif, France.

†R. A. Miller, D. G. Maloney, R. Warnke, R. Levy, *N. Engl. J. Med.* **306**, 517 (1982).

although this has not been a significant problem so far. Two patients may have had allergic reactions, but recovered. The others have experienced few or no side effects when they were treated with monoclonal antibodies. Nevertheless, antibodies made by the patient react with the monoclonal antibody and could prevent it from reaching the tumor cell target.

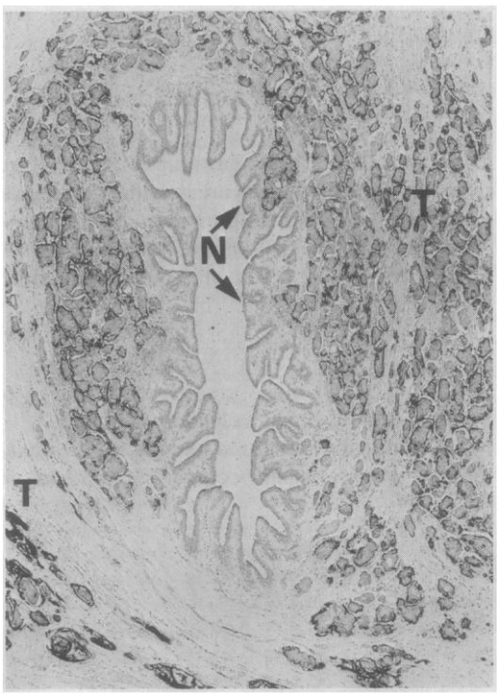
According to Koprowski, potential problems with allergic reactions might be circumvented if a single large dose of the monoclonal antibody could deliver enough of a cell-killing agent to wipe out the tumor. Usually a single exposure to foreign antigen only sensitizes the recipient's immune system; it is the next exposure that causes trouble.

Schlossman and Ritz are exploring another strategy for using monoclonal antibodies in cancer therapy that does not pose the danger of inducing allergic reactions. The treatment is currently being tried on patients with acute lymphocytic leukemia (ALL) who have not been helped by conventional therapy. It permits administration of higher than usual doses of chemotherapeutic agents and radiation doses that would ordinarily be fatal because they destroy the bone marrow.

To prevent this, a portion of the patient's bone marrow is removed, treated with a monoclonal antibody and complement to destroy leukemic cells, and then frozen. After the radiation and drug treatments are completed, the patient is implanted with his own bone marrow. "Within weeks." says Schlossman, "they begin to reconstitute their lymphoid and myeloid systems. They become hematologically indistinguishable from normal."

It is still too early to tell whether this therapy is superior to other second-line treatments for ALL. Patients with this leukemia, whose initial therapy has failed, may live about 2 years if they are treated with additional drugs. Of the three patients given the new therapy, one has remained disease-free for 15 months and the second for 13 months. The third relapsed after 6 weeks. "The results are still anecdotal," Schlossman explains, "but we are enthusiastic enough to look for more patients."

Because of the concern about the use of foreign proteins for cancer therapy, a major priority for future research is the development of human monoclonal antibodies against tumor-associated antigens. Schlom points out, "It is obvious that if you have your choice, you would want a human monoclonal antibody to minimize the immune reaction."



Mammary tumor

The normal cells (N) forming the large mammary duct in the center of the micrograph are unstained by the monoclonal antibody to a mammary tumor–associated antigen, whereas the infiltrating tumor cells (T, dark color) react with the antibody. [Source: Jeffrey Schlom, National Cancer Institute]

One stumbling block is the lack of an easy-to-grow human myeloma line that does not produce antibody of its own. There are mouse myeloma lines that meet these criteria, largely as a result of the work of NCI's Michael Potter, but no human lines. Cancer Institute officials hope to be able to budget up to $0.5 million to remedy this deficiency.

A better myeloma line is only half the solution to the problem of making human monoclonal antibodies, however. Human cells that make antibodies to tumor antigens are also needed. Appropriate antibody-producing cells can be obtained from mice by injecting them with tumor cells and using the spleens as a source of B cells, an approach clearly not possible with humans.

But there may be a way around this, according to Schlom. "The hypothesis," he explains, "is that human tumor antigens are shed and picked up by the lymph nodes. B lymphocytes there may be primed to make antibodies against those tumor cells."

Schlom and David Wunderlich of NCI have fused lymphocytes from lymph nodes removed during surgery for breast cancer with mouse myeloma cells. The resulting hybridomas make human antibodies against breast tumor antigens. But, many of them, like other human-mouse hybridomas, are unstable. They lose their human chromosomes and stop making the human antibodies. Nevertheless, a few of the hybridomas were stable and the results suggest that the approach is feasible. "You just have to try harder," Schlom says, "to get stable lines."

The development of appropriate human monoclonal antibodies is a technical problem that is likely to be solved. More serious difficulties are related to the nature of the tumors. Not all tumors of a given type are alike in the antigens they carry, as can be shown by their reactivity with monoclonal antibodies. Schlom, David Colcher, and Patricia Hand of NCI have prepared a series of monoclonal antibodies against antigens associated with human breast tumors. They determined the reactivity of five of them to tumor tissues taken from 45 patients. Only nine of the tissues reacted with all five and some reacted with only one.

A further complication is the heterogeneity of the cells within a single tumor and of their surface antigens. For example, Schlom showed that a given antibody does not bind to all the cells in a breast cancer and the Minna group obtained similar findings with lung cancers. According to Paul Bunn, one of Minna's colleagues at NCI, "There is a tremendous amount of heterogeneity. Clearly, if you are thinking about antibodies in terms of delivering therapy it is a major problem." Mixtures of monoclonal antibodies may be needed to ensure that all the cells of a tumor are wiped out.

Research on the clinical use of monoclonal antibodies is still very young. Although many investigators are hopeful that they can develop a successful cancer therapy with the agents, no one expects this to happen easily or without delays. Meanwhile, researchers all emphasize that the antibodies are providing tools with which to approach some of the fundamental problems of cancer cell biology.—JEAN L. MARX