Antibodies Made to Order

Chimeric antibodies—which are part mouse and part human—may help solve the problems hindering the therapeutic use of monoclonal antibodies

The discovery of monoclonal antibody technology once again raised visions of finding "magic bullets" that could specifically seek out and destroy cancer cells without harming normal cells. Although early clinical trials with the antibodies have shown some signs of success, they have also pointed up a problem. Clinicians have had to use monoclonal antibodies of mouse origin because it has proved very difficult to produce human monoclonals of appropriate specificity in sufficient quantities for therapy. As foreign proteins, the mouse antibodies often evoke counteracting immune reactions that may destroy their effectiveness and may also cause allergic side effects in the patients.

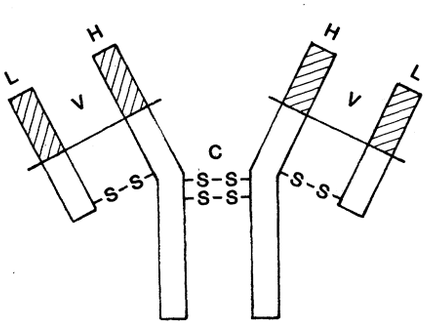
For example, Ronald Levy and his colleagues at Stanford University School of Medicine recently reported that 5 of 11 leukemia patients showed evidence of an immune reaction against the mouse monoclonal antibodies used to treat their disease (*1*). These individuals experienced little benefit from the experimental therapy, although the remaining patients showed at least transient improvement.

A new method of making antibodies may help to solve this antigenicity problem. Antibody-producing cells normally assemble complete genes for the proteins by combining three or four separate DNA segments, a procedure which helps to generate the vast array of antibodies needed to protect the body against foreign invaders. By merging monoclonal antibody and recombinant DNA technologies, immunologists have taken nature's already formidable antibody-synthesizing capabilities a step further. They can produce novel antibody proteins by combining portions from one antibody gene with segments of another, thereby designing molecules to have whatever features the investigator wants. "The type of antibodies that can now be made are limited primarily by the imagination of the investigator," says Sherie Morrison of Columbia University College of Physicians and Surgeons.

Among the more interesting of the new constructions are chimeric antibodies in which the antigen-recognizing variable regions of mouse antibodies are joined with the constant regions of human antibodies. These antibodies may be less likely to produce an immune response in humans than the all-mouse monoclonals, although this has not yet been shown.

Potential applications of chimeric antibodies include, in addition to cancer therapy, the treatment of autoimmune conditions such as multiple sclerosis and systemic lupus erythematosus. The idea here is that the antibodies may be able to destroy the specific immune cells needed for mounting the abnormal attack on the body's own tissues. In a related fashion, the antibodies may also eventually be used for immune suppression of individuals who require organ transplants. Last but not least, the new technology should help in dissecting out the contributions of the various regions of the antibody proteins to their functions.

The key development that allowed the production of designer antibodies occurred about 2 years ago. Three groups of investigators, one including Morrison and Vernon Oi who was then at Stanford University School of Medicine, another from Marc Shulman's and Nobumichi Hozumi's laboratories at the University of Toronto, and a third including David Baltimore of the Whitehead Institute and the Massachusetts Institute of Technology and his collleagues, independently devised methods for introducing antibody genes into myeloma cells, a type of tumor cell that grows well in culture.



In a chimeric antibody, both the light and heavy chains consist of mouse variable regions (shaded) joined to human constant regions.

This made possible an essential step in the new technique. Once the desired recombinant antibody genes are constructed, they must be transferred into cells that will translate them into protein structure. Antibody genes are very choosy in this regard. They carry control sequences that restrict their expression to a certain kind of immune cell. Myeloma cells, which are derived from antibody-producing cells, not only express antibody genes efficiently but also have the appropriate machinery for adding the necessary carbohydrate residues to the proteins and eventually secreting the completed antibodies into the culture medium.

Morrison and Oi, the Toronto group, and also Michael Neuberger and his colleagues at the MRC Laboratory of Molecular Biology in Cambridge, England, have since gone on to make various chimeric and other designer antibodies (*2*, *3*, *4*). Antibody molecules contain four protein chains, two copies of a light chain that has a molecular weight of about 25,000, and two copies of a heavy chain with a molecular weight of about 50,000. Each chain is subdivided into a variable and a constant region. The variable regions of the two heavy and two light chains of a complete antibody form its antigen-recognition site and differ from one antibody to the next. The constant regions are the same for all antibody chains of the same class.

Construction of the gene for a chimeric antibody protein requires joining the DNA coding for a variable region of the desired specificity with the DNA of a constant region. To obtain a complete chimeric molecule, such recombinant genes must be made for both light and heavy chains. The two genes are then introduced into the same myeloma cells. Synthesis and assembly of the antibody in the cells proceeds normally, producing functional molecules with the expected specificities.

Although this procedure sounds cumbersome, all of the techniques are fairly standard. Once the variable and constant region DNA sequences are in hand, chimeric antibodies can be produced in 2 to 3 months, Morrison estimates. Constant region sequences of human origin are readily available; genes representing the two classes of light chains and the five major classes of heavy chains have been cloned in a number of laboratories.

Obtaining human variable region sequences of appropriate specificities for the proposed applications is more difficult. The human antibody genes that have already been cloned are not likely to be useful for the cancer therapy, for example. For this application the variable region must specifically recognize a

tumor-associated antigen. It should be possible to generate antibodies that react with potential therapeutic targets by monoclonal antibody technology, at least in the mouse system. "There is a tremendous flexibility of the mouse system to generate an antibody with a defined specificity," Morrison notes. "The limitation is that it's always a mouse molecule."

Human monoclonals have been difficult to obtain because the standard method for making the antibodies entails injecting the target antigen into a subject—usually a mouse—that can provide appropriate antibody-producing cells. These cells are then harvested from the spleen of the immunized animal and fused with myeloma cells to produce hybridomas. Those that secrete the desired antibodies can then be identified and maintained in culture. This procedure can obviously not be used to produce human monoclonals.

Investigators have tried to circumvent the problem by obtaining antibody-producing cells from the blood of individuals who have already been exposed to an antigen, such as cancer patients who have the ability to make antibodies against tumor antigens. Although the hybridomas produced with these cells may make human antibodies of the desired specificity, they are often unstable and stop producing the antibody.

Consequently, the current plan is to generate the variable region sequences in the mouse monoclonal system and then join them with human constant regions. These chimeric antibodies should be less antigenic than the mouse monoclonals in humans, provided that the general supposition that the immune attack is primarily directed against the constant region of the antibodies is correct.

The immune system is capable, however, of making antibodies, called anti-idiotype antibodies, against variable regions—even of its own antibodies. This is thought to be part of the system that normally regulates antibody synthesis. It remains to be seen whether this will be a problem with the chimeric antibodies. Nevertheless, Shulman points out that immunologists find it "not so easy" to make anti-idiotype antibodies in experimental animals when they deliberately set out to do so.

In general, investigators are optimistic about the therapeutic potential of chimeric antibodies, a potential that will no doubt receive a great deal of attention from the biotechnology industry. "I have great hopes for the chimeric approach," says Oi, who is now at the Becton-Dickinson Monoclonal Center in Mountain View, California. "We may not eliminate the antigenicity, but we will certainly decrease it. Then the question is—will we decrease it enough to be useful?" Early clinical trials to test the antigenicity of chimeric antibodies in humans are expected to get under way shortly in a number of laboratories.

Although the possible use of chimeric antibodies for the therapy of cancer and autoimmune conditions has received the most attention, Shulman points out that the antibodies might also find an application as a preventive for infectious diseases, such as hepatitis B. This disease is very common, especially in the Far East, and is associated with an increased risk of liver cancer.

Women who carry the hepatitis B virus can transmit it to their children at the time of birth. A few years ago, investigators showed that they could prevent the development of the carrier state in infected newborns. They first treated the infants with antibody against the hepatitis virus for the first month or two when the babies' immune systems were not developed sufficiently to mount a response of their own. Then they gave a hepatitis B antigen as a vaccine.

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This treatment has not been widely used because antibody to hepatitis B virus has had to be prepared from human blood and only small quantities are available. In any event, avoiding the administration of blood-derived products to infants may be desirable if there is also a chance, however slight, of transmitting other diseases.

A step toward obtaining a designer antibody that could be used for this application may have already been taken by Tasuku Honjo and his colleagues at the Osaka University Medical School (*5*). They have recently reported the construction of a gene for an antibody heavy chain in which the constant region is of mouse origin and the variable region is of human origin and is directed against the hepatitis B surface antigen.

Another problem with monoclonal antibody technology, which can be circumvented by the chimeric approach, is that the investigator cannot control which class of antibody will be produced by the hybridomas. There are five major classes, the IgA's, IgD's, IgE's, IgG's and IgM's (for immunoglobulins A, D, E, G, and M). The class into which a particular antibody falls is determined by the constant region of the heavy chain.

The functions of the classes differ. A particular type of antibody might be suitable for one application but not another. For example, the IgG's play a major role in neutralizing foreign antigens in the bloodstream. In contrast, IgE's trigger many of the reactions that produce allergic responses. There are indications that, under appropriate conditions, IgE's might also be used to block allergic responses. This class of antibodies is not ordinarily made by hybridomas, but can by made by the chimeric approach as Neuberger and his colleagues have demonstrated (*6*). The ability to mix and match antibody constant and variable regions generally makes it possible to construct antibodies of any class and any specificity.

In addition to the proposed medical applications, Neuberger suggests that chimeric antibody technology can be applied to protein purification. He and his colleagues have used it to make dual-function molecules that consist of antibody variable regions joined to enzyme proteins. "If you make such a construct," Neuberger explains, "the hybrid is secreted from mammalian cells and is easily purified on an antigen column." The two enzymes purified so far retained their activities after this treatment. Both of them consist of just one protein chain that readily refolds to its native configuration. It might be more difficult, he points out, to apply the technique if an enzyme consists of more than one protein or is less able to resume its native configuration.

Dual-function molecules of this type might also be useful for immunoassays. The antibody portion would recognize the antigen under investigation and the enzyme portion would catalyze a reaction that produces a detectable product, thus permitting determination of the antigen concentration. Finally, joining the antigen-combining portion of an antibody to a toxin protein might produce a molecule that could destroy cancer cells, still another variation on the long-sought magic bullet.—**JEAN L. MARX**

References:

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