"Magic" monoclonals

Only in the era of Herceptin have antibodies begun to fulfill Ehrlich's dream of "magic bullets" from blood serum.

BY RICHARD A. PIZZI

Ever since Paul Ehrlich proposed, a century ago, that antibodies might be used as "magic bullets" to target and destroy human diseases, scientists have sought to tap the biotherapeutic potential of these natural proteins. Yet only in the past few decades has the therapeutic promise of antibodies begun to be realized, as advances in immunology and animal cell culture led to a technique for the development and production of monoclonal antibodies, an achievement that undoubtedly ranks as one the foremost advances in 20th-century medicine.

Monoclonal antibodies may be coming closer to those magic bullets that immunologists dreamed about, but to appreciate what the future holds, one must understand some of the history of 20th-century immunology and the notable achievements in cell culture that led biomedicine to this juncture.

When challenged by foreign substances (antigens), the human immune system recognizes and responds by producing high-affinity binding proteins (antibodies) that can interact with and clear the antigens. This property has been known for more than a century, and it has led scientists to utilize the immune system to generate therapeutic agents for a variety of settings.

Polyclonal antibodies have been used clinically since the 1890s, when horse- and sheep-derived antibodies were introduced as serum therapy to treat diphtheria, measles, and scarlet fever. While these therapies proved effective, their widespread use has been limited because of the immune response generated in humans by animal-derived proteins. Serum contains a "cocktail" of polyclonal antibodies and other proteins, and human immune systems invariably react against some of the harmful proteins, causing fevers, rashes, joint pain, and sometimes anaphylactic shock. Hence, researchers have long sought a "humanized" monoclonal antibody that would not sicken patients and could be grown indefinitely.

César Milstein, left, and Georges Köhler in Kenya, 1979.

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The theoretical underpinning of an immunological revolution was provided by Niels K. Jerne (1911–1994). In the mid-1950s, Jerne explicated his natural selection theory of antibody formation, which proposed that the capacity of the immune system to recognize myriad antigens was predetermined, existing in the body prior to contact with a foreign substance. These natural antibodies develop during fetal life in the absence of external stimuli. The antigen selects the antibody molecule that has the best fit, and the binding of antigen to antibody stimulates the production of a particular antibody through growth of the particular cells. Jerne's theory contradicted previous understandings of antibody response (known as "instruction theories"), which held that the antigen served as a template for the production of antibodies.

In later research, Jerne explained how the human immune system matures, developing from stem cells to mature lymphocytes, and how immune system response is regulated. His theories greatly stimulated subsequent immunological research and have been applied to the diagnosis and treatment of disease. So confident was Jerne of the accuracy and utility of his work that he predicted in 1969 that all the interesting problems of immunology would soon be solved and that nothing would remain except the details associated with the management of disease! While his prognostications were a bit premature, no one doubts the seminal influence of Jerne's theoretical work.

Subsequent immunologists who sought the magic bullet that polyclonal antibodies did not provide found help in Jerne's theories and in the development of animal tissue culture. Tissue culture as a research technique arose at the beginning of the 20th century as a method for studying the behavior of animal cells free of the systemic variations that might arise in an animal under the stress of an experiment. The term "tissue culture" is used generically to refer to organ culture and cell culture, although the name derives originally from the study of undisaggregated fragments of tissue. However, most research in this area since the 1950s has used dispersed cell cultures. The development of tissue culture as a modern, sophisticated technique owes much to the technical improvements made possible by the commercial supply of reliable media and sera and by the greater control of contamination with antibiotics and clean-air equipment.

Benefiting from the advances in modern tissue culture and inspired by the search for magic bullet antibodies, two scientists at the University of Cambridge (U.K.) devised a technique that would be judged the most important methodological advance in the field of biomedicine during the 1970s,

and one of the most significant of the 20th century. Georges Köhler and César Milstein discovered a way to produce monoclonal antibodies in vitro with relative ease, fusing normal antibody-producing cells with tumor cells to produce long-lived cell lines.

Partners at Cambridge

Köhler and Milstein were an unlikely pair. César Milstein (1927–2002) was born and raised in Argentina, earning a doctorate in Buenos Aires and another Ph.D. at Cambridge in Britain. Forced to resign from the Argentine National Institute of Microbiology because of the political persecution of liberal intellectuals, Milstein returned to Cambridge in the early 1960s and shifted his research from enzymology to immunology. Georges Köhler (1946–1995) was German by birth, but he performed his doctoral research at the Institute for Immunology in Basel, Switzerland, where Niels Jerne was director. In 1974, he joined Milstein's laboratory in Cambridge, fascinated by the ongoing research into the mutation of antibodies. Although Milstein was almost 20 years older than Köhler, the two scientists worked as partners on the cell fusion project that would change immunology.

The cells responsible for producing antibodies lack the capacity to survive and reproduce for a long period of time if they are removed from the body and placed in a tissue culture. Like any other cells, these antibody-producing cells (B cells) can become cancerous and form tumors. These tumors are called myelomas. But cancerous cells have unlimited growth potential, an insidious ability in most circumstances. Köhler and Milstein found a way to combine the reproductive capacity of myeloma cells with the predetermined antibody specificity of normal immune cells in a process called somatic cell hybridization. The result was astounding—the production of what they termed a "hybridoma". They had, in essence, created monoclonal antibodies.

To create a hybridoma, Köhler and Milstein culled spleen cells from mice that were immunized with a selected antigen and then fused them with myeloma cells maintained in culture in the laboratory. A hybrid of these two cell types can survive and continue to divide. The myeloma cells contributed the capacity for survival, whereas the spleen cells directed the synthesis of antibodies with a preselected specificity. The resultant hybridoma cells could be maintained indefinitely in culture vessels or grown in mice. The hybridoma culture thus produced monoclonal antibodies for as long as necessary. Köhler and Milstein were awarded the 1984 Nobel Prize in Physiology or Medicine for their discovery of the "principle of production of monoclonal antibodies." Niels Jerne was a co-recipient of the prize, noted for his influential theories concerning "the specificity in development and control of the immune system."

Monoclonal antibodies are used widely as diagnostic and research reagents. For example, monoclonals are used to identify different forms of tumors and to follow the development of tumors. They can also distinguish between different types of white blood cells, an essential process in the characterization of certain immune deficiency conditions. Monoclonals can also help diagnose disease caused by infectious agents that need to be identified with specificity. The use of monoclonals as therapeutic agents has proceeded more slowly and with limited, but growing, success.

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Perhaps the most famous therapeutic use of monoclonals involves Herceptin, a drug that offers hope to some breast cancer patients. Developed in the late 1980s by oncologist Dennis Slamon of the University of California, Los Angeles, and by the biotech company Genentech, Herceptin binds the HER-2 protein, a growth factor receptor found on tumor cells in 25–30% of women with breast cancer. Herceptin was approved by the FDA in 1998 and is the only commercially available monoclonal drug thus far that seems to be effective against solid tumors. Another monoclonal antibody is rituximab, or Rituxan, developed by Genentech and IDEC Pharmaceuticals. It is a drug that binds to the CD20 molecule found on most B-cells and is used to treat B cell lymphoma. Other monoclonals approved for human treatment are OKT3—the first monoclonal drug, used to prevent the acute rejection of organ transplants—and abciximab, a monoclonal that inhibits the clumping of platelets and helps prevent reclogging of arteries in patients who have undergone angioplasty.

Antimouse antibodies

Although the transition from discovery to applied medical use has proven successful in selected cases, relatively few monoclonals have been used for human therapy. The primary problem is that the human immune system views mouse antibodies as foreign and mounts an immune response to them. The results are human antimouse antibodies (HAMAs), which eliminate the therapeutic antibodies and form immune complexes that damage the kidneys. Researchers now use genetic engineering or chimeric techniques with monoclonal drugs such as Herceptin to alter the mouse proteins to contain humanized amino acid sequences, which minimizes the rejection immune response.

Biomedical scientists are constantly redesigning monoclonal antibodies for use in humans, and many cancer researchers think that the future of monoclonal antibodies is in combinatorial treatment, in which antibodies are used in conjunction with chemotherapy or surgery. Regardless of its current imperfections, the desire to find a magic bullet against disease will likely lead monoclonal antibody research to flourish in the 21st century.

Further reading

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