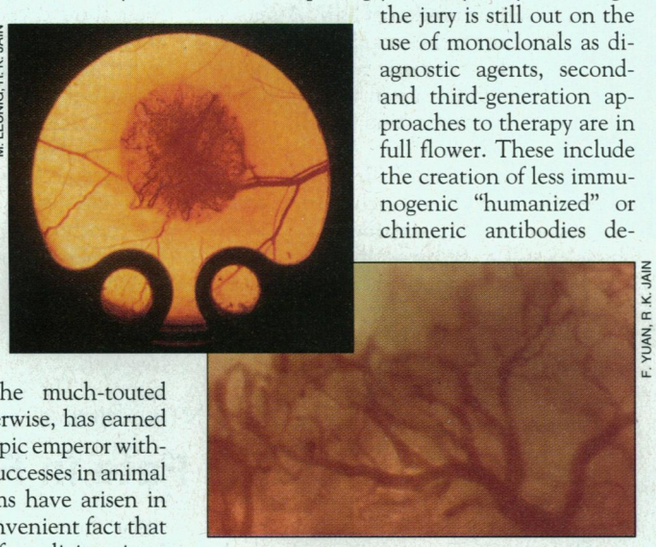
IMMUNOLOGY

# Monoclonal Antibodies at Age 20: Promise at Last?

It has been 20 years since César Milstein and Georges Köhler, working at the Laboratory of Molecular Biology in Cambridge, England, stunned the world of biology by announcing the discovery of monoclonal antibodies. By marrying the exquisite specificity of antibodies to the tireless manufacture of these proteins by immortal mouse myeloma cells, monoclonals promised a revolution in human diagnostics and therapy. Whether used as unadorned "naked" antibody or armed with radioisotopes or toxins, these agents tantalized biologists—and venture capitalists—with their potential to home in on tumor cells and eradicate malignancies. Inevitably, they were rechristened "magic bullets," and a 1984 book by that title modestly heralded "the most exciting adventure in the annals of modern medicine."



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**In a twist.** Convoluted blood vessels in tumor impede delivery of therapeutics, including monoclonals. The vessels themselves are a target of one type of monoclonal.

Two decades later, the much-touted monoclonal, naked or otherwise, has earned the reputation of a microscopic emperor without clothes. Despite many successes in animal models, significant problems have arisen in humans, not least the inconvenient fact that mouse-derived antibodies often elicit a vigorous human immune response that in some cases limits therapy to one-time doses. Such problems prompted tumor immunologist Lloyd J. Old, director of the Ludwig Institute for Cancer Research, to remark, "*In vivo veritas* should become the motto for the field." And monoclonals have apparently lost some of their luster on Wall Street, too: Eli Lilly & Co., which paid $350 million for the monoclonal biotech company Hybritech in 1986, dumped it last month, reportedly at a garage-sale price of less than $10 million.

Given this recent history, it was not surprising that a tone of apologia and contrition crept into many a talk at a conference last month, sponsored by the Cancer Research Institute in New York,* assessing future directions in the field. As meeting chair Old acknowledged in an overview that put past enthusiasms in sobering perspective, "The difficulties were underestimated, the timeline was unrealistic, and the claims were overstated. Add to this the pressure of keeping financial investors excited, and you have a sure formula for disappointment and disillusionment." Or, as Jean-Pierre Mach of the University of Lausanne observed succinctly, "Some people were selling the skin of the bear before killing it."

And yet, for all the full-dress pessimism on display, the meeting—the first of what is intended to be a biannual series—managed to throw out the bath water while hanging onto a surprisingly healthy baby. Although the jury is still out on the use of monoclonals as diagnostic agents, second- and third-generation approaches to therapy are in full flower. These include the creation of less immunogenic "humanized" or chimeric antibodies designed to reduce reactions in patients, attempts to identify better targets in tumors and associated cells, and the use of monoclonals to disrupt growth signals to the cancer cell. Moreover, several therapies appear on the verge of establishing enduring clinical success, especially against hematologic cancers such as lymphomas.

**Big guns.** The promising story of the anti-B1 antibody, first isolated in Stuart Schlossman's lab at the Dana-Farber Cancer Institute in Boston in 1980, suggests that even the old-fashioned approach can be made to work—given enough time. This antibody targets the CD20 antigen, a cell surface protein that is expressed on about 90% of B cell lymphomas but does not appear on either primitive B cells or mature, differentiated B cells. Oliver Press of the University of Washington summarized impressive results with this monoclonal over the past 8 years against non-Hodgkin's lymphoma, a B cell cancer of the immune system that will strike approximately 51,000 people in the United States this year.

In the late 1980s, the Seattle group began testing several different monoclonal antibodies linked to radioactive iodine-131, in a Phase I trial designed to measure the therapy's toxicity. The subjects—19 patients whose disease had relapsed—were treated with varying doses of radiolabeled anti-CD20, and every patient had a measurable response, Press reported. In 16 of them, all evidence of disease vanished, and blood tests showed that CD20-positive B cells, including the cancerous ones, had been eradicated. Eight of those 16 have remained free of disease from 3 1/2 to 8 years. In a recently completed Phase II trial, to test the efficacy of the anti-CD20 monoclonal at the maximum tolerated dose, Press said 17 of 21 patients receiving the treatment experienced complete responses.

Press admits that the bullet in this treatment is more radical than magical. Patients receive relatively high doses of radiation from the iodine-131 and as a result must undergo a bone marrow transplant following treatment to overcome the effects of radiation. The entire procedure requires 4 weeks of hospitalization. "Our bias," Press says, "is that in order to get cures and long-lasting remissions, we're going to need high doses."

There may, however, be room for low-dose approaches, as Mark S. Kaminski and co-workers at the University of Michigan Medical Center have begun to demonstrate. The Ann Arbor group has also focused on non-Hodgkin's lymphoma. In a recently completed Phase I trial in patients who had failed chemotherapy, doctors pretreated patients with unlabeled, naked anti-CD20 antibody to "fill sinks," such as the liver and spleen, where antibodies tend to get sequestered before they reach tumor tissue; they then administered a one-time dose of "hot" anti-CD20 antibody. Twenty-two of 28 patients responded, including 14 complete responses; the response rate in patients whose tumors had become resistant to chemotherapy was about 70%. Median duration of complete responses exceeds 15 months, and because the radiation dose was one-seventh that of the Washington regimen, patients required only a 3-day hospital stay. "The main toxicity, other than reversible bone marrow suppression," Kaminski says, "was boredom."

**New targets.** Another strategy by which clinicians are boosting the effectiveness of monoclonal antibodies is choosing new antigens to target. Blocking an antigen that happens to be a growth factor receptor sitting on the surface of a tumor cell, for example, could deprive cancer cells of crucial growth signals and disrupt the cascade of biochemical changes that trigger cells to divide. That is precisely the approach pursued by John Mendelsohn and colleagues at the Memorial

* Monoclonal Antibodies and Cancer Therapy: The Next Decade, 16–18 October, New York.

Sloan Kettering Cancer Center in New York. They have begun a clinical trial using a chimeric mouse-human antibody (225 IgG1) that binds to the epidermal growth factor (EGF) receptor. This receptor is overexpressed on numerous tumors, and Mendelsohn is testing the hypothesis—suggested by preclinical experiments—that a combination of antibody and traditional chemotherapy will have a synergistic effect that enhances cell killing.

Napoleone Ferrara of Genentech described a similar effect using monoclonals to block the signal that induces the formation of blood vessels in tumors. Vascular endothelial growth factor (VEGF) is in all endothelial cells of the vasculature, but the level of expression found in nearly all tumors, Ferrara says, "is orders of magnitude higher than in normal tissues." In animal experiments using rhabdomyosarcoma, a muscle fiber tumor, an antibody that blocked VEGF resulted "in a dramatic suppression of tumor growth that is dose dependent," Ferrara reported. In a subsequent experiment, the Genentech group tested the combination of antibody and cisplatin against the same tumor. "When we combined the chemotherapy with the monoclonal antibodies," Ferrara says, "there was really a remarkable regression."

One new twist on the strategy of using monoclonals calls for changing the target from tumor cells to seemingly innocent bystanders. Wolfgang Rettig of the German pharmaceutical company Boehringer Ingelheim recommended attacking stromal cells, fibroblasts that occupy a kind of buffer zone between capillaries and the tumor tissue proper. Unlike normal resting fibroblasts, these cells churn out growth factors, extracellular matrix proteins, and other proteinaceous excretions that suggest they have somehow been activated and recruited to the service of the neighboring tumor.

Rettig's group serendipitously discovered that a monoclonal named F19 interacts with a highly specific antigen on the surface of these activated fibroblasts. Dubbed "fibroblast activation protein" (FAP), it appears to be expressed normally in certain fetal cells and newborn children, during wound-healing, and in stromal cells surrounding solid tumors. In a recent experiment done in conjunction with workers at Memorial Sloan Kettering, Rettig and his colleagues infused 17 patients whose colon cancers had spread to the liver with radiolabeled anti-FAP to test its ability to home in on tumor sites. The labeled antibody clearly identified the site of liver metastases in 14 of the 17 patients, including two whose tumors didn't show up on computerized tomography scans. Rettig noted that FAP is expressed in about 90% of lung, breast, colon, and pancreatic tumors. Targeting it with a barrage of monoclonal antibodies might provide a new avenue of attack. "By going after the stroma," he said, "you have new opportunities."

**Smarter bullets.** Several other papers presented at the meeting suggest that the early "naive" belief in antibodies has matured into more ambitious biological engineering. Take the approach described by Carlos F. Barbas III of the Scripps Institute. Along with colleague Richard Lerner, Barbas has developed a technology for synthesizing human antibodies that involves identifying the active region of antibody binding and then creating a huge library of up to 1 billion genetic variations in the binding region, cloning these variants, and then screening each one for high-affinity binding of the target protein. The Scripps group has used this approach to create up to 1 billion variations in two regions of an antibody that binds to the gp120 surface protein of HIV. After several cycles of mutagenesis and selection, they ended up with a synthetic human antibody with a 420-fold increase in binding affinity and a much-increased binding half-life, on the order of a week.

Even if the ideal antibody is constructed, Rakesh K. Jain of Massachusetts General Hospital reminded everyone of formidable obstacles still to be overcome, especially in the treatment of solid tumors. Using video micrography and a novel system of "transparent windows" that allows direct observation of human tumors grown in immunodeficient mice, Jain demonstrated how the unusual physiology of tumors thwarts even the most innovative therapy—vessels feeding tumors are contorted by sharp bends, shunts, and loops; immune effectors like white blood cells rarely pass through; and blood flow occasionally shuts down or even reverses itself. Moreover, Jain says, therapeutic agents—whether antibodies, T cells, or other large molecules—must cross the vessel wall to reach tumor cells, driven in part by higher blood pressure inside the vessels than out. But studies have shown that in tumors, unlike normal tissue, the hydrostatic pressure outside in the tumor tissue is as high as that inside the vessel, creating a pressure barrier.

Despite many remaining obstacles, researchers who have stuck with the technology remain upbeat about monoclonals as they enter their third decade. "Our methods are still crude," says Press. "We probably have this tool that's going to be useful, and we may not know the best way to use it yet. I think people feel apologetic that the field did not deliver on public expectations, but I always thought that it would take a long time to satisfy the expectations raised in the popular press, so I was neither surprised nor disappointed. I think we're making slow, steady progress."

**–Stephen S. Hall**

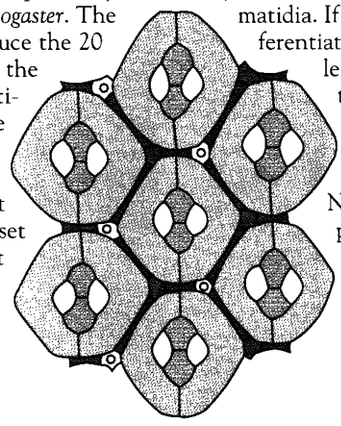
*Stephen S. Hall is a science writer in Brooklyn, New York.*

DEVELOPMENTAL BIOLOGY

# Sifting Mitosis, Cell Fate in Fly Eyes

In the riot of cell divisions that gives rise to complex organs and tissues, each cell must be assigned its specific form and task. But exactly how cell division and fate determination are related in different organisms is one of the oldest unsolved mysteries in developmental biology. Take the compound eye of the fruit fly *Drosophila melanogaster*. The cell fate decisions that produce the 20 specialized cells in each of the eye's approximately 800 retinal units, or "ommatidia," are preceded by two waves of cell division (mitosis)—raising the possibility that these divisions somehow set the genetic "switches" that enable the ommatidium cells to respond to developmental signals from their neighbors. Without a precise way to manipulate the mitotic waves, however, researchers have not been able to test this possibility directly—until now, that is.

By inserting a human gene into fruit flies, a pair of researchers at the Massachusetts General Hospital Cancer Center in Charlestown has blocked the second episode of cell division in the developing fly eye. The cells produced in this spurt of mitosis normally specialize into light receptors, pigment and cone cells, sensory bristles, and other key parts of the ommatidia. If cell division is crucial to differentiation, blocking mitosis would leave the flies without many of these crucial cell types. But that's not what developmental geneticists Iswar Hariharan and Joriene de Nooij found, as they report on page 983. Undifferentiated cells left over from early cell divisions specialized to produce all the cell types, although the resulting eyes were still abnormal because they did not have enough of some cells. The lesson, says Hariharan, is that "the pattern of division the cells have got to go through ... is irrelevant for programming cell fates."



**Complex vision.** Ommatidia are arrayed regularly in a normal fly eye, shown here. When the second mitotic wave is blocked, the supply of cells runs out before the pattern can be completed.

SOURCE: T. WOLFF AND D. READY IN *THE DEVELOPMENT OF DROSOPHILA MELANOGASTER*, COLD SPRING HARBOR LAB. PRESS, 1993