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

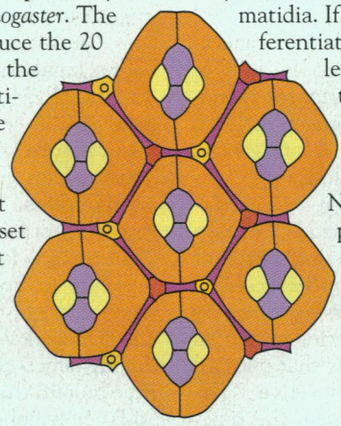
Stephen S. Hall is a science writer in Brooklyn, New York.

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

Developmental biologist Larry Zipursky of the University of California, Los Angeles, describes the work as "a beautiful set of results." And he and other researchers say this picture, in which cell division simply provides a large pool of undifferentiated cells that can then be "recruited" to develop into cells with specialized roles, may prove to be a general paradigm for development in multicellular creatures. "In a lot of systems in which cell division is followed by cell type determination, similar mechanisms may be operating," says Kevin Moses, a developmental geneticist at the University of Southern California.

Hariharan and de Nooij had long been intrigued by the fact that all but the first five photoreceptor cells in the ommatidium acquire their fates after the second of two waves of mitosis that sweep over the fruit fly eye. "The only way we could ask what role this mitotic division has in programming specialized states," Hariharan says, "was to stop it." To accomplish this feat, de Nooij and Hariharan targeted expression of a human mitosis-inhibiting protein called p21 to cells poised to undergo the second mitotic wave.

When the researchers abolished the wave in this way, they found that those cells that hadn't already differentiated to produce the first five photoreceptor cells went on to differentiate normally, producing the additional photoreceptors and other cells of the ommatidia. The eyes ended up with a clumpy, disordered appearance, however, because they simply didn't have enough of the building blocks needed to complete many of the 800 ommatidia.

Because the work rules out the possibility that the second mitotic wave is necessary for subsequent cell differentiation in the eye, de Nooij and Hariharan's experiment "enormously simplifies how we can think about cell fate determination in the eye," says Donald Ready, a developmental biologist at Purdue University. "It appears as though it is simply the local environment that communicates to the cell what it should become."

Tracking down those local influences is the goal of ongoing research in many laboratories. But the transgenic flies may also offer clues to another puzzle: the genes that regulate cell division in the fruit fly eye. Geneticists can now screen the transgenic *p21* flies for mutations that make their gnarled eyes either better or worse. Genes found to blunt or amplify *p21*'s effects are likely to be promoters or inhibitors of the cell cycle, Hariharan explains. Combined with the demonstration that some *Drosophila* eye cells acquire their fates independently of mitosis, the work could help solve an old mystery about young organs.

–Wade Roush

AIDS RESEARCH

New Clues Found to How Some People Live With HIV

Nearly 15 years ago, before it was possible to screen for the AIDS virus, an infected gay man donated blood in Sydney, Australia. In the next 3 years, no fewer than seven people received transfusions of products containing his HIV-contaminated blood—seemingly a tragedy in the making. But as more than a decade passed, the expected tragedy failed to materialize. Neither the donor himself nor any of the transfusion recipients appear to have been harmed by this HIV.

Now investigators have uncovered a possible explanation for this anomaly that may shed light on a long-standing AIDS mystery—why a few "long-term nonprogessors" (LTNPs) can live normally with HIV while most infected people sicken and die. It may also bolster an unpopular strategy for developing an AIDS vaccine that might altogether prevent a lethal HIV infection.

Harmless HIV? Nicholas Deacon finds a defective virus in some LTNPs.

On page 988, a research team from three Australian institutions reports results suggesting that the members of the Sydney group have not developed the immunodeficiencies of AIDS because they were infected with a particularly weak strain of the virus. Specifically, the team, led by molecular biologist Nicholas Deacon of the Macfarlane Burnet Centre for Medical Research in Victoria, Australia, found that the virus is missing parts of the *nef* gene, which has been shown in other studies to be needed for full-scale viral replication.

This is not the first time that a defective *nef* gene has been linked to an LTNP. In the 26 January issue of the *New England Journal of Medicine* (*NEJM*), Ronald Desrosiers of the New England Regional Primate Research Center and colleagues reported a similar finding in HIV isolated from a hemophiliac LTNP. But, says Desrosiers, the number of cases in the Sydney Bloodbank cohort makes it "more significant and more dramatic."

Indeed, Anthony Fauci, head of the National Institute of Allergy and Infectious Diseases (NIAID), says the Sydney cohort provides a unique opportunity for studying nonprogression. "It's a very important experiment of nature," says Fauci, whose own lab is also studying LTNPs. "It nails down the concept that one of the reasons people might be [LTNPs] is due to defective virus." He cautions, however, that a defect in the virus is "certainly" not the only explanation. His group and others have found that immunologic factors, such as the presence of strong antibodies that can neutralize the virus and potent killer cells that can clear infected cells, appear to account for other cases of nonprogression.

Deacon and his colleagues came to their conclusion after sequencing HIV isolates from four of the eight Australians. (Two of the recipients had died of non–AIDS-related diseases, and the researchers have not been able to isolate HIV from the other two.) All four had large deletions of the *nef* gene, as well as defects in other genetic elements that may have crippled the virus. But the researchers are particularly interested in the *nef* defect as a possible cause of nonprogression because of previous animal studies.

Work done by the Desrosiers group and others, for example, suggests that Nef, the protein produced by the *nef* gene, signals HIV-infected cells to make more copies of the virus. The lack of an intact *nef* may therefore account for the fact that members of the Sydney cohort have low HIV concentrations in their blood. And in 1991, Desrosiers and co-workers reported in *Cell* that deleting *nef* from SIV, HIV's simian cousin, effectively disarms that lethal virus. "What our study adds is that the same applies for HIV in humans as applies to SIV in macaques," Deacon says.

Still unclear, however, is how common *nef* gene defects are in LTNPs. Virologist David Ho, director of the Aaron Diamond AIDS Research Center, says the Deacon team's paper clearly shows that *nef* deletions can weaken HIV, but "it's not the usual explanation for LTNPs." Indeed, Ho and coworkers reported, also in the 26 January *NEJM*, that they found no evidence of gross *nef* defects in any of 10 LTNPs studied.

But even if *nef* defects aren't a major cause of nonprogression, there's another reason the Australian results may be important: They might influence AIDS vaccine development. Three years ago, Desrosiers showed that in addition to being nonpathogenic, *nef*-deleted SIV is a powerful vaccine, protecting monkeys from subsequent infection with deadly SIV. He suggested then that it