Animal Cells Transformed in Vivo

Researchers at Duke University have developed what they believe is a versatile and simple method of inserting novel genes into the somatic cells of live animals: shoot them in with a biological version of a BB gun. Conventional means for transforming such cells are complex, indirect endeavors. Typically, the cells targeted for transformation are drawn out of the organism, transformed with a retrovirus, and then reintroduced. Indeed, in what will be the first clinical trial for human gene therapy later this year, National Institutes of Health researchers will use that approach with bone marrow cells to correct adenosine deaminase deficiency (ADA) in a select group of patients (*Science,* 8 June, p. 1182).

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But at the 13 June meeting of the Tissue Culture Association in Houston, Stephen A. Johnston of Duke described his simplified technique. He uses a redesigned version of the so-called gene gun, first developed at Cornell University, which sold the rights to it to DuPont last year. The gene gun shoots DNA-coated, gold microprojectiles into living cells. The gun was first used successfully to transform algae and yeast in 1988 and is now used routinely to transform plants. In April, for example, Monsanto used the gene gun to transform the recalcitrant crop, corn.

But the gene gun, as it was originally designed, used a gunpowder-like explosion to shoot the DNA into cells, and it damaged the more delicate animal cells. The new model uses high-pressure gas to propel the DNA. "We can shoot tiny BBs at high velocity, with little trauma to the tissue," Johnston says. Indeed, Johnston and his collaborators at Cornell and Dupont have just shown that the redesigned gun can insert novel genes into the ear, skin, and surgically exposed liver cells of mice.

Johnston is confident that the redesigned gene gun, also known as a biolistics device, for biological and ballistic, can be developed for medical uses as well. "Our goal is to design an instrument to do transformations on somatic cells in a surgical setting," says Johnston. "We have it down to almost a hand-held instrument. We are trying to keep the idea simple." What's more, he adds, "the versatility of the gun allows us to transform various tissues." Muscle or skin cells might be modified to provide circulating factors, such as insulin, says Johnston. Or the endothelial cells in the blood vessels of heart attack victims might be engineered to express the clot-buster TPA, tissue plasminogen activator. But first, Johnston admits, he and other researchers must demonstrate that this technique can be used to achieve stable expression of inserted genes and that it can transform an adequate number of cells.

Within the last few months other researchers have also announced new techniques for inserting genes into live animal cells. Christine E. Holt of the University of California, San Diego, inserted genes into the neuroepithelium of frog embryos using lipofectin, a synthetic lipid that entraps DNA much as liposomes do. And Jon A. Wolff of the Waisman Center at the University of Wisconsin injected genes into the skeletal muscle of young mice (see *Science*, 23 March, p. 1465). But Wolff's technique didn't work well with brain, blood, liver, or spleen tissue. Thus, neither of these techniques appears as versatile as the gene gun.

How have Johnston's colleagues reacted to the news of the first successful use of the gene gun in live animal cells? Much as plant biologists did earlier, says Johnston. "People say, 'It is a bizarre system. But it works.' " ■ ANNE SIMON MOFFAT

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workers are now trying to determine whether the adhesion molecule initiates plaque formation by attracting the immune cells. At the very least, Gimbrone says, the new adhesion molecule should serve as a marker for early detection of atherosclerosis, and if it is involved in plaque initiation, it may also provide a point of attack for preventing the buildup of the artery-clogging deposits.

However macrophages get into plaques, they may have several effects in addition to stimulating the proliferation of arterial smooth muscle cells by secreting lymphokines. According to Daniel Steinberg of the University of California, San Diego, they may also contribute to the cholesterol deposition that is one of the hallmarks of typical atherosclerosis lesions.

Macrophages are notorious for producing strong chemical oxidizing agents such as the superoxide anion. That helps them kill foreign bacteria and clean up debris at inflammatory sites. But it can also have a less desirable effect, Steinberg says, namely, the oxidation of LDL (low-density lipoprotein) cholesterol, known as the bad form of cholesterol because it promotes atherosclerosis.

Oxidized LDL cholesterol not only attracts more macrophages to a growing atherosclerotic site, but it is also taken up by the cells much more readily than native LDL cholesterol. As a result the site becomes clogged with cholesterol-laden macrophages, leading to further progression of atherosclerosis.

Finally, LDL cholesterol is not the only blood lipid that is a risk factor for heart attacks. Epidemiological studies have shown that lipoprotein(a) is a risk factor, too, although how the molecule might predispose to heart attacks has been unclear. New findings from Ralph Nachman's lab at Cornell Medical College in New York City now suggest that it may work by interfering chemically with endothelial defenses against clot formation, much as the damage caused by angioplasty interferes physically.

About 2 years ago, researchers learned that the protein component of lipoprotein(a) has a structure similar to plasminogen's. That suggested that the lipoprotein might somehow contribute to blood clot formation. Nachman now reports that it may do this by interfering with plasminogen binding to the endothelial lining. As a result of the inhibition of binding, he says, the release of plasmin from tissue-bound plasminogen goes down 80 to 90%, and "over a long period, this may contribute to atherogenesis." Indeed, when Nachman and his colleagues looked for lipoprotein(a) deposition on the endothelium of coronary arteries, they found it on diseased arteries, but not on normal ones.

The reactions that contribute to the development of arterial lesions, both the ordinary atherosclerotic type and those seen in transplanted hearts and after balloon angioplasty, are by and large the same reactions that the body uses to defend itself against injury and invading pathogens. As atherosclerosis expert Russell Ross of the University of Washington School of Medicine in Seattle points out, for example, "Atherosclerosis is wound-healing gone wrong." A good deal of evidence now indicates that an intact endothelium is needed to keep those reactions from going wrong. ■ JEAN MARX