MUTANT MIMICRY

Throwing a monkey wrench into the genetic machinery of AIDS

By RICK WEISS

Imagine a terrorist organization seizing a U.S. munitions factory and producing lethal weapons for use against our allies. In a sense, this is what the AIDS virus (HIV) does when it commandeers a white blood cell's genetic machinery to produce a new arsenal of immune-cell-destroying HIV.

Now imagine that the original factory workers, before fleeing for their lives, secretly reprogram one of the machines to make a defective component. The crippled parts get incorporated into every weapon made by the invaders. When the terrorists begin their next attack, they find themselves militarily impotent.

AIDS researchers in several U.S. laboratories are experimenting with a comparable strategy of biomolecular sabotage. The procedure, a form of gene therapy known as intracellular immunization, would involve genetically altering most or all of a person's white blood cells, spurring the cells to manufacture a constant supply of mutant viral components. Any invading AIDS virus attempting to mass-produce copies of itself inside such a cell would inadvertently incorporate many of these flawed pieces into its progeny, rendering the new generation unable to carry on the HIV reproductive cycle.

No one has yet attempted intracellular immunization in humans, and clinical trials remain at least a few years away. But laboratory experiments using cultured cells suggest the approach may prove effective as a treatment for people infected with HIV, or even as a peremptory "vaccination" for individuals at high risk of acquiring the infection.

Indeed, of all the diseases that might someday succumb to gene therapy, those that primarily affect blood cells — as AIDS does — offer the greatest hope of success, researchers say. That's because all blood cells derive from common precursor cells in the bone marrow, and gene-wielding scientists can tinker with bone marrow cells with relative ease.

"The approach is really worth working toward," says Didier Trono of the Whitehead Institute for Biomedical Research in Cambridge, Mass., who last year helped engineer a line of cultured monkey cells containing a mutant HIV gene that inhibits HIV replication within those cells. "Even though from a technical point of view it seems difficult," he adds, the scientific advances needed to implement the strategy in humans will probably become available "sooner than one thinks."

In the past seven months, at least three research teams have published results confirming the potential value of intracellular immunization or closely related methods as a defense against AIDS.

Michael H. Malim, Bryan R. Cullen and their colleagues at the Duke University Medical Center in Durham, N.C., inserted into cultured monkey cells a viral gene coding for a mutant HIV protein. In its normal form, the protein, called Rev, is critical for the production of other HIV proteins in infected cells. In its mutant form, Rev interferes with viral replication, apparently by competing with normal Rev for critical binding sites inside an infected cell. HIV-infected cells producing the mutant protein showed significantly reduced HIV replication, the researchers report in the July 14, 1989 CELL.

In that same issue, Maurice Green and his colleagues at the St. Louis University School of Medicine describe a related approach. They used cultured human cells containing a mutant version of another HIV protein called Tat. In its normal form, Tat helps initiate viral replication in HIV-infected cells. Since cells readily absorb the tiny protein from the surrounding culture medium, the researchers simply added mutant Tat to their cultures. Once absorbed by the cells, the altered protein interfered with viral reproduction there.

This approach has potential advantages over true intracellular immunization, Green says, because it wouldn't require permanent alteration of a patient's cellular DNA; theoretically, physicians could deliver mutant Tat as a pill.

On the other hand, small proteins such as Tat make less-than-ideal drugs, Green notes. For example, they are vulnerable to attack from various protein-cleaving enzymes both inside and outside cells. So scientists would have to synthesize versions of Tat similar enough to normal Tat to compete with the real McCoy, yet different enough to interfere with its proper function *and* altered in some way that enables it to resist enzymatic destruction. Despite these challenges, Green says, the approach remains "scientifically very exciting."

In Trono's approach — one of the most encouraging efforts to date — he and his colleagues engineered a line of cells that produce a constant supply of a mutant HIV protein known in its normal form as Gag. HIV was largely unable to replicate within cells expressing the mutant Gag protein, the researchers report in the Oct. 6, 1989 CELL.

The Gag protein has advantages and disadvantages as a focus for gene therapy, Trono notes. On the positive side, Gag proteins normally combine with each other into large clumps called "multimers" before playing their part in viral replication. Experiments indicate that it takes only a few defective Gags within a multimer to disrupt the multimer's function. Thus, moderate levels of mutant protein appear sufficient to block HIV replication. This is helpful, says Trono, because one of the major bottlenecks in gene therapy is the challenge of inducing engineered cells to churn out large amounts of protein in their new environs.

On the down side, however, Gag's role in viral reproduction comes late in the replication process, when viral components have already been produced and are ready for final assembly. "Gag is a late player in the HIV life cycle," Trono says, and with scientists still unsure what causes an HIV-infected cell to die, a roadblock at the Gag stage may come too late to prevent cell death.

Further experiments should help scientists determine which HIV genes show greatest potential as targets for intracellular immunization. Research may also show that, rather than permanently altering a patient's bone marrow cells, gene therapists could periodically provide transfusions of engineered blood cells — a simpler procedure researchers have begun to investigate using a bacterial gene at the National Institutes of Health in Bethesda, Md. (SN: 9/23/89, p. 197).

In the meantime, molecular biologists seek to improve upon today's relatively inefficient methods of injecting genes into blood cells — another factor limiting immediate application of human gene therapy.

But perhaps the greatest obstacle lies outside the lab: Researchers, bioethicists and federal officials have yet to agree upon experimental protocols that adequately address all the scientific and ethical implications of inducing permanent genetic changes in humans. Gene therapy has stirred considerable controversy, but it may find a more sympathetic audience as it nears potential application to the AIDS epidemic. □