GENE THERAPY

Putting Antibodies to Work Inside Cells

A team of researchers at the Dana-Farber Cancer Institute in Boston has turned the immunology world inside out—or in this case outside in. Normally, antibodies ply their trade only after exiting a cell's interior. But by using a combination of antibody engineering and gene therapy, the Dana-Farber team, led by immunochemist Wayne Marasco, has directed an antibody to a specific location *inside* a cell. The achievement, described in the 16 August *Proceedings of the National Academy of Sciences*, raises the possibility of using antibodies within cells to block the construction of viruses or harmful proteins, such as the oncoproteins whose activity contributes to the uncontrolled growth of cancer cells. Adding to the intrigue, the antibody Marasco and his colleagues put into cells was able to interfere with the assembly of the AIDS virus, thereby reducing both its cell-killing potential and its infectivity.

The new technique has been tried only on mammalian cells in lab cultures, but it is already drawing some rave reviews. "To have [antibodies] work inside a cell and not at the cell surface is novel and very interesting. Conceptually, it opens up the whole possibility of doing intracellular targeting," comments immunologist Norman Letvin of the New England Primate Research Center.

Indeed, the dream of enticing cells to make intracellular antibodies has been an alluring one for more than a decade. "Many a postdoc has gone to his death working on it," says University of Michigan gene therapist Gary Nabel. The reason for the frustration? Once antibodies are made they are normally transported right to the outer cell surface or they are secreted into the bloodstream. What gave Marasco the hope of succeeding was the relatively new ability of molecular immunologists to tailor-make antibodies by assembling their genes from different bits and pieces of DNA. "There's really been an explosion in antibody engineering over the last 5 years," he says.

Marasco and his colleagues started with an antibody called F105 that they knew binds to gp120, a crucial HIV envelope protein that the AIDS virus uses to attach to and infect its target cells. By piecing together DNA, the researchers created a gene for a shaved-down version of F105 that consisted mainly of the amino acids forming the site where the normal antibody binds gp120.

The investigators also needed to make sure that F105 and the envelope protein had a good chance of meeting, so they concocted a way to direct the antibody made by their engineered gene to the endoplasmic reticulum (ER), a membranous compartment in the cell interior where the HIV envelope protein starts life as part of a larger precursor designated gp160. To do that, Marasco and his colleagues added still more DNA to the gene they were creating, attaching a so-called leader sequence, which would cause the antibody to be synthesized only in the ER. The hope was that if F105 was produced there it would bind to the gp120 segment of the gp160 precursor and prevent the envelope protein from being formed. The fear, however, was that F105 would be made in the ER and be quickly whisked out of the cell like other antibodies. So the researchers engineered a second version of their F105 gene to include a "retention signal," a few extra peptides designed to anchor the antibody in the ER. They then put the engineered genes separately into cultured cells with one of the standard plasmids used for gene transfer.

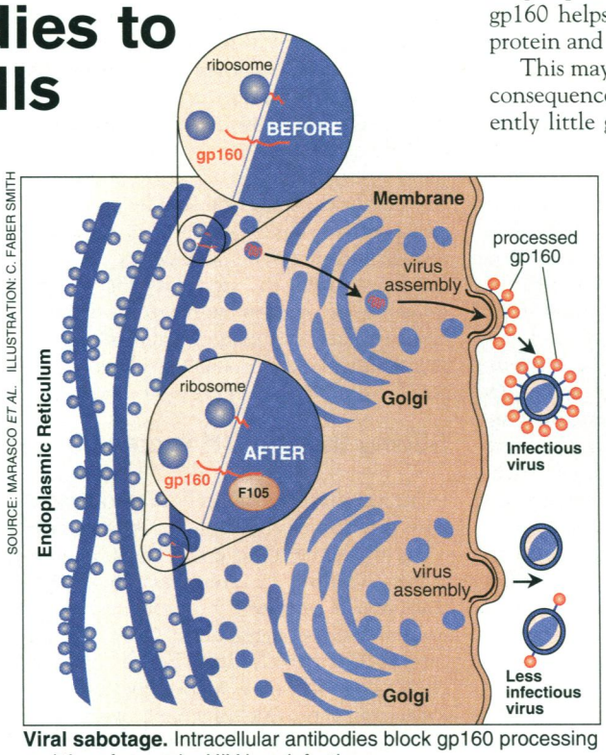
To Marasco's surprise, once the genes were inside cells, that extra piece of engineering proved unnecessary and even undesirable, since it seemed to yield an unstable molecule. In contrast, their simpler antibody lingered in cells just fine. The molecule is apparently held within the ER because it attaches itself to another ER protein called BiP. Assays by the Dana-Farber team showed that the antibody does indeed pair up with gp160, and as a result the production of gp120 is dramatically lowered. Marasco says his group believes that antibody pairing with gp160 helps the ER degrade the precursor protein and thus gp120 is never formed.

This may have therapeutically beneficial consequences. For one thing, with apparently little gp120 on their surfaces, the altered cells proved much less capable of fusing with other cells to form the cell-killing syncytia that some researchers think are at least partly to blame for the immune cell loss of AIDS. Moreover, the virus particles released from the antibody-producing HIV-infected cells proved more than 1000 times less infectious than ordinary HIV. Also encouraging from a therapeutic point of view: Production of the intracellular antibody appeared to have no toxic effects on the cells.

This novel antibody approach is a long way from application in AIDS patients, however. For a start, researchers must find a way to get engineered antibody genes into all cells that are infected with HIV—and only into those cells. That's a much more difficult task than simply popping a gene into cultured cells in the lab. "The problem with the next step is the problem with any gene therapy—delivery," says Letvin. Moreover, the F105 antibody only weakens the AIDS virus and doesn't stop its production entirely. The next step may be to direct antibodies against HIV proteins like Tat and Rev that are needed for virus replication. But there's a major hurdle before that can be accomplished. Investigators are still puzzling out how to retain antibodies in locations other than the ER. Tat and Rev, for instance, are made in the cytoplasm. "If you hit those targets, you can really stop the virus. But it's another major technological leap to get antibodies to function in the cytoplasm," admits Marasco.

While Marasco is confident that scientists will someday be able to steer intracellular antibodies to almost any part of the cell, the existing technology should keep him and others more than busy. Beyond a possible approach to AIDS therapy, the technique may hold promise for cancer therapy. The obvious targets: any of several oncoproteins, like ErbB, whose abnormal activity has been linked to cancer. "There's already a large number of oncoproteins that travel through the endoplastic reticulum. We can hit them now," says Marasco. If that prophecy proves as accurate as his ability to guide F105, then antibodies may prove as important inside the cell as outside.

–John Travis



SOURCE: MARASCO *ET AL.* ILLUSTRATION: C. FABER SMITH

Viral sabotage. Intracellular antibodies block gp160 processing and therefore make HIV less infectious.