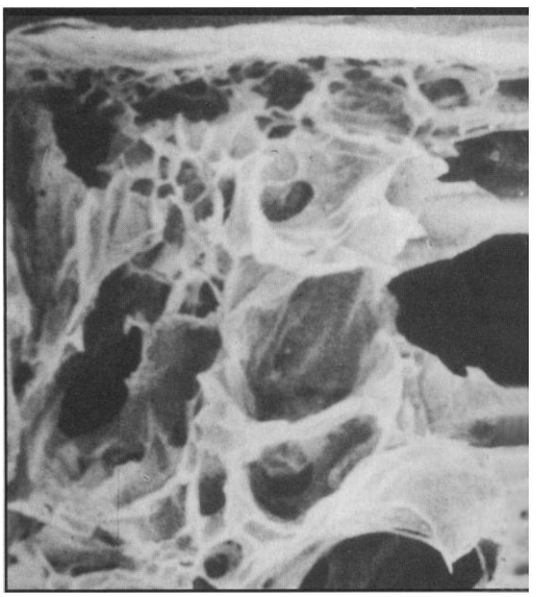
A Genetic Shield to Prevent Emphysema?

Lymphocytes programmed to produce alpha-1 antitrypsin may protect lungs from the ravages of emphysema



A deadly lung. *Without a protective shield of alpha-1 elastase. Alpha-1 antitrypsin deficiency is one cause of*

Genes In Medicine

"It is," says Ronald G. Crystal, "the 'Star Wars' approach to gene therapy."

Crystal, chief of pulmonary medicine at the National Heart, Lung, and Blood Institute, envisions putting the gene for a protein called alpha-1 antitrypsin into an aerosol that could be sprayed into the lungs of people with emphysema. Because of an absence of the alpha-1 gene that occurs in a significant percentage of emphysema victims, these people have lungs full of holes where tissue has been destroyed by a natural compound, neutrophil elastase that ravages the delicate alveolar walls of the lower respiratory tract.

Like a Star Wars defense, Crystal says, alpha-1 is a shield that protects the lungs from being eaten away by neutrophil elastase. In a healthy individual, the concentration of alpha-1 in the lungs is sufficient to keep the neutrophil elastase at bay. And it is the absence of an alpha-1 shield that makes victims vulnerable to emphysema and, occasionally, to a genetically related disorder that results in a bleeding liver.

Crystal thinks that alpha-1 antitrypsin deficiency is an ideal candidate for human gene therapy one of these days. One reason: There is an enormous amount of information about the disease that can be used to design a genetic approach. At a meeting on genetics last summer at the Jackson Laboratory in Bar Harbor, Maine, Crystal described alpha-1 antitrypsin deficiency as the "only genetic disease that we thoroughly understand at the molecular level." And, although a lot of work still needs to be done before he and his colleagues would be ready to submit a protocol to the NIH's Recombinant DNA Advisory Committee (RAC), which has jurisdiction over this research, a pretty reliable blueprint is already in hand.

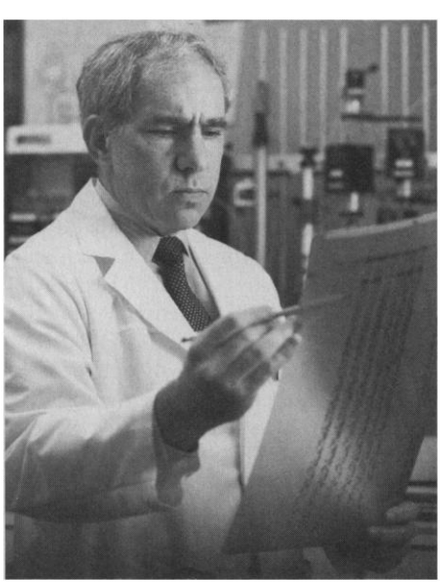
The incidence of the disease is well defined. Alpha-1 antitrypsin deficiency generally occurs in Caucasians of Northern European decent: it is rarely seen in Blacks or Asians.

The alpha-1 gene is located on chromosome 14. Although the gene is expressed in three cell types, most alpha-1 antitrypsin is made in the hepatocytes of the liver. In general, a mutation in the gene will result in the secretion of too little alpha-1, Crystal reports, which in turn means that the alpha-1 shield in the lungs can be penetrated by neutrophil elastase. (Disease in the liver is usually caused by a slightly different mutation that manifests itself by an accumulation of alpha-1 in hepatocytes.)

The mechanism by which emphysema progresses in alpha-1 deficient patients is also well detailed. By the time someone is 30 years old, there are likely to be preliminary signs of disease: shortness of breath, for instance. Cigarette smoking makes things worse because, Crystal says, "the Met^{358} residue at the active site of alpha-1 is vulnerable to oxidation by free radicals. In turn, alpha-1's already compromised ability to inhibit neutrophil is compromised further." Eventually, breathing becomes almost impossible and as the patient's pulmonary function deteriorates, there is less oxygen in the blood; vital organs throughout the body suffer "oxygen starvation."

Few patients live past 60.

With all this knowledge, can alpha-1 antitrypsin deficiency be treated by conventional therapy? The answer is "probably yes," says Crystal. For instance, in the past few years, Crystal and his colleagues have also worked out a strategy for infusing alpha-1 prepared from pooled plasma from normal donors. Patients received weekly infusions, and the researchers reported in the 23 April 1987 issue of *The New England Journal of Medicine* that protective levels of alpha-1 could be detected in the lung. Since then, hundreds of infusions have been administered, with no evidence of adverse reactions beyond fever in just a few patients.

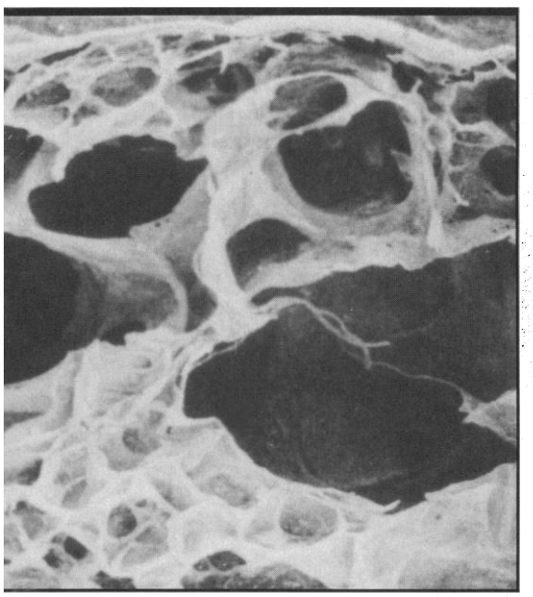


Ronald G. Crystal. *Planning a "Star Wars" defense with designer lymphocytes.*

On the basis of data showing that alpha-1 does get into the lung in what ought to be therapeutic concentrations, the Food and Drug Administration has approved its use for emphysema patients, even though there are as yet no data to show that the treatment prolongs life. "Emphysema is just too slow a disease to get that kind of information," Crystal says. "It will take 20 years." The treatment is costly: weekly infusions could add up to $30,000 a year.

All this would seem to rule out emphysema as an early candidate for gene therapy, because current guidelines limit the as-yet untried technique to diseases that are untreatable by other means. But Crystal argues that knowledge gained from treating emphysema patients with alpha-1 have laid the basis for a more radical approach: an attempt to deliver cells carrying the alpha-1 gene directly to the lungs. "So much of the biology and safety data are in," he says.

Crystal is hoping to insert the alpha-1 gene in T lymphocytes, which would then be delivered to the airways in an aerosol. At the meeting of the American Society for Clinical Investigation last spring, Crystal



antitrypsin, lungs are ravaged by the body's own neutrophil emphysema.

reported success, using the N2 retrovirus as a vector, in getting lymphocytes to express and secrete human alpha-1 in mice.

In a separate experiment reported in the August *Annals of Internal Medicine*, he showed that human alpha-1 can be aerosolized and that, when sprayed into the lungs of emphysema patients, it produces a strong alpha-1 Star Wars shield against neutrophil elastase.

Many challenges remain. One of them is finding a virus vector that could withstand being aerosolized. "I doubt the N2 vector could take it," Crystal speculates. But he bets that a form of the common cold virus, which thrives by being sprayed around, might do the trick. "We know that these viruses penetrate cells," he says, "and they may be effective at carrying therapeutic genes"—as long as their cold-causing capacity can be inactivated.

If alpha-1 antitrypsin deficiency were to yield to this form of genetic medicine, Crystal guesses that other genetically related pulmonary diseases might not be far behind: chronic bronchitis, for example, or cystic fibrosis, a lethal deficiency for which the gene has just been cloned (*Science*, 8 September, p. 1059).

Much of this is speculation, to be sure, but it is not far out. As one researcher who works with retroviruses at the molecular level told *Science*, "We may well be at the point now where the next big leaps will come in genetic medicine, with the Ron Crystals of the world using genes and their products therapeutically, while the rest of us continue to plug away at the ultimate repair of a defective gene itself."

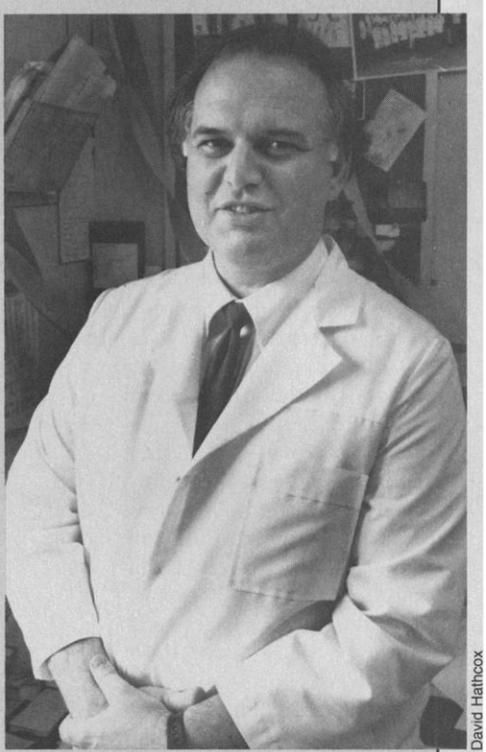
■ BARBARA J. CULLITON

ADA Deficiency: A Prime Candidate

When researchers began giving serious attention to gene therapy about 5 years ago, a rare disorder known as adenosine deaminase, or ADA, deficiency was at the top of the list of diseases that fit the criteria for initial human studies. It is a single gene defect that in theory can be corrected by modifying a gene to produce the missing enzyme. It is also a serious—in this case lethal—disease for which ideal alternative treatment does not exist.

Today, researchers still view ADA deficiency as a prime early candidate for gene therapy. But they are no longer talking about replacing the defective gene. Instead, they are contemplating stitching ADA-producing genes into lymphocytes, which would then be transfused into a patient. The engineered cells would, it is hoped, manufacture the missing ADA.

The ADA gene plays an important role in the development of the B and T cells of the immune system. Children born with a defective gene for ADA fail to develop a healthy immune system, largely because the lack of ADA leads to the build-up of a metabolite that is selectively toxic to T and B lymphocytes. As a result, ADA-deficient kids are susceptible to infection by every bacteria and virus that comes along. They fail to thrive and die early in childhood.



David Hathcox

Lymphocyte engineer. *R. Michael Blaese is hoping to transfuse patients with lymphocytes containing ADA-producing genes.*

Three years ago, Michael Blaese, French Anderson, and their colleagues at the National Institutes of Health "cured" ADA deficiency in a test tube.* They used a retroviral vector called SAX to carry a cloned ADA gene into cultured B and T lymphocytes from kids with ADA deficiency. The gene not only made ADA, but it made it in sufficient quantities to suggest that it would deliver a therapeutic dose to a human patient.

In its purest theoretical form, gene therapy for ADA deficiency would entail repairing the defective gene in the pleuripotent stem cells of the bone marrow from which B and T immune lymphocytes come. However, as noted earlier, stem cell therapy has so far eluded researchers' grasps.

Blaese, who is currently treating immune-deficient children, is ready to try to correct the problem one step removed—by giving patients lymphocytes that carry a normal ADA gene. During the past 3 years, he has been successful in culturing lymphocytes from ADA patients. In the lab, everything is ready. The excitement that Blaese's colleagues feel at getting the cell lines to grow and express ADA is palpable. So is the frustration at not being able to move as quickly as they would like into human trials.

The next step is to submit a protocol to the NIH's Recombinant DNA Committee, or RAC, which must approve all human gene therapy proposals.

ADA deficiency is an extremely rare disease—fewer than ten children are born with it each year in North America. Within the past couple of years, some of these infants have been given a polyethylene-glycol–modified form of ADA, but after a few doses its effectiveness is often lost, for reasons that are not understood. It is these kids that Blaese and Anderson would like to treat first. Blaese estimates that infusions of ADA-carrying lymphocytes would have to be given every 3 months or so. A protocol is being drafted. Blaese says, "I'm almost ready to go." ■ B.J.C.

*Philip W. Kantoff *et al.*, "Correction of adenosine deaminase deficiency in cultured human T and B cells by retrovirus-mediated gene transfer," *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6563 (1986).