Getting to the Heart of Genetic Disease

New developments in understanding and treating genetic diseases, in particular those affecting the heart, lungs, or blood, were the focus of a recent symposium on the "Genetic Basis of Human Disease: Molecular Mechanisms and Strategies for Therapy." The symposium, which was sponsored by the National Heart, Lung, and Blood Institute, was held on 30 November and 1 December of last year at the Bethesda campus of the National Institutes of Health. A few highlights follow.

New Treatment Found for Hereditary Emphysema

Researchers have shown that they can correct the biochemical defect that causes the continuing lung degeneration of patients who have a hereditary form of emphysema. The assumption is that this will halt the progress of the disease and prolong the patients' lives, although the corrective treatment has not been available long enough to know this for certain.

The development of the new therapy, which was described at the genetic disease symposium by Ronald Crystal of the National Heart, Lung, and Blood Institute (NHLBI), is the product of a better understanding of what causes the lungs of emphysema patients to deteriorate. Individuals with the hereditary form of the disease, who constitute about 2% of the 2 million emphysema patients in the United States, have a defective gene for a protein called α1-antitrypsin.

This protein binds to protein-digesting enzymes, such as trypsin, and inhibits their action. The name α1-antitrypsin is something of a misnomer, because the prime target of the inhibitor in the body is not trypsin, but is instead another protein-degrading enzyme known as elastase.

Elastase is part of the disease-fighting armament of the white blood cells that are normally present in small numbers in the lungs to help protect against respiratory infections. α1-Antitrypsin ordinarily prevents elastase from damaging the lung tissue itself, but as a result of the genetic defect, not enough of the inhibitor reaches the lungs and the damage caused by elastase gradually builds up. Symptoms usually become apparent after about one-quarter of the lung surface has been affected. People who have defective α1-antitrypsin genes can start showing emphysema symptoms in their twenties and thirties, and usually die in their fifties.

Crystal and his colleagues have now shown that weekly intravenous injections of α1-antitrypsin can maintain effective concentrations of the inhibitor in the blood and lungs of these patients. Once lung damage occurs, it cannot be reversed, but the injections may be able to keep it from getting worse.

The treatment is expensive. It costs about $30,000 a year, Crystal estimates, with about two-thirds of this going to pay for the α1-antitrypsin.

The NHLBI workers have not yet demonstrated that the new therapy will prolong patients' lives. Crystal estimates that this will take 5 to 10 years, and the first patients were treated in 1986. The U.S. Food and Drug Administration has nonetheless approved α1-antitrypsin therapy for patients with the genetic form of emphysema on the basis of the demonstration that it corrects the biochemical defect underlying the disease.

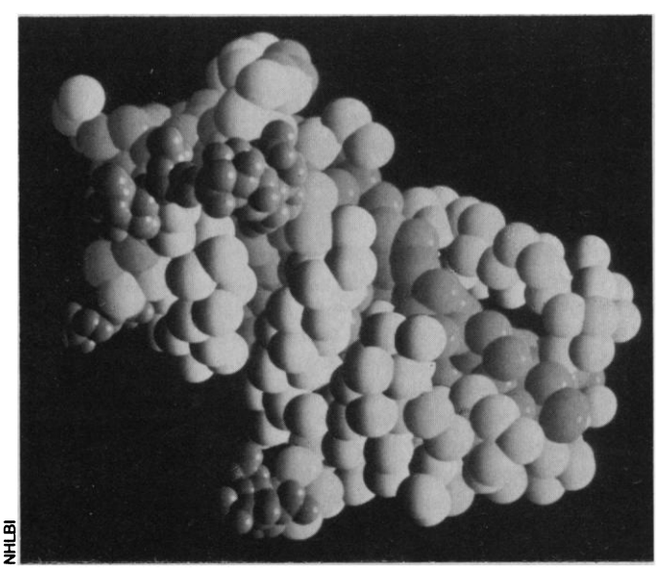
The agency is requiring, however, that a national registry of the patients receiving α1-antitrypsin be kept to establish the efficacy and safety of the therapy. The 250 patients treated so far at the NHLBI and elsewhere have received α1-antitrypsin that has been prepared from human plasma. According to Crystal, these individuals have experienced no harmful side effects, except for transient fever in a few instances. The α1-antitrypsin preparation is heated to inactivate viruses, such as those causing hepatitis and AIDS. "We've never seen viral contamination," Crystal says.

α1-Antitrypsin that is produced by recombinant DNA technology is also available now, but cannot be given by injection. The recombinant inhibitor, which is made in bacteria, lacks the attached carbohydrates that are present on the mammalian protein. The absence of the carbohydrates causes the protein to be excreted in the urine, instead of being delivered to the lungs where it is needed.

Recombinant α1-antitrypsin is effective as an elastase inhibitor, however, and the researchers are now working to develop an aerosol system for delivering it directly into the lungs. This may also help to reduce the cost of the therapy by reducing the amount of α1-antitrypsin that needs to be administered.

Another possibility for the future is the use of gene therapy to permanently correct the α1-antitrypsin gene defect. Crystal and his colleagues have shown that they can insert the cloned human α1-antitrypsin gene into mouse fibroblasts, which subsequently make the inhibitor protein in culture and, after transplantation, in nude mice. The human protein can be found in the blood and lungs of the mice.

In addition, Crystal notes, in some patients with defective α1-antitrypsin genes, the inhibitor accumulates in liver cells, its normal site of synthesis, instead of being transported by the blood to the lungs. As a



NHLBI

The α1-antitrypsin molecule. *In this computer simulation of the inhibitor the large balls represent amino acids and the smaller, darker balls represent attached carbohydrate. The NHLBI workers generated this α1-antitrypsin image from data produced in Robert Huber's laboratory at the Max Planck Institute for Biochemistry in Marteinsreid, Germany.*

result, some of these individuals suffer liver damage severe enough to warrant liver transplants. They would be good candidates for gene therapy if methods for correcting the α1-antitrypsin gene within liver cells become available. Techniques for doing this do not currently exist, but investigators are on their way to developing them (*Science*, 14 October 1988, page 191).

Whether any of these current or potential therapies will also prove applicable to the vast majority of emphysema cases, which are caused by smoking, is currently unclear. Cigarette smoke promotes the development of emphysema at least partly, however, by its effects on α1-antitrypsin.

Not only does cigarette smoke attract more elastase-producing white blood cells to the lungs, but it also contains chemicals that oxidize a particular methionine residue in α1-antitrypsin, rendering the inhibitor incapable of binding to elastase and preventing its action. "In a sense," Crystal says, "when you smoke cigarettes you are giving yourself the equivalent of a genetic disease."

The NHLBI group is now determining whether the α1-antitrypsin in smokers' lungs recovers after they stop smoking. Such recovery might be expected, and in that event α1-antitrypsin therapy would apparently provide no extra benefits. Nevertheless, as Crystal points out, "A proportion of emphysema patients who stop smoking continue to go downhill."

Finally, the NHLBI workers have shown that they can replace the vulnerable methionine residue of α1-antitrypsin with other amino acids that are not oxidizable without destroying the protein's ability to inhibit elastase. This raises the possibility that treatment with the modified α1-antitrypsin could be used to protect the lungs of people who fail to quit smoking. But even if this worked for emphysema, it would not protect against the other deleterious effects of smoking, such as an increased risk of heart attacks and cancer.

Lipoprotein(a)'s Role in Heart Attacks Explored

The recent cloning of a gene encoding a key protein constituent of lipoprotein(a) has provided a major clue that may help to explain the role of this lipid-carrying blood particle in promoting heart attacks. According to Richard Lawn of Genentech, Inc., of South San Francisco, Angelo Scanu of the University of Chicago, and their colleagues, sequencing of the cloned gene revealed that part of the protein that it encodes, which is called apolipoprotein(a) [apo(a)], resembles a portion of another protein called plasminogen.

"There was this exciting and unexpected homology to plasminogen," Lawn said at the symposium, "but does this suggest anything about [apo(a)] function?" It might.

Lipoprotein(a) is one of the carriers of cholesterol in the blood. It is less well known than some of the other cholesterol carriers. The low-density lipoproteins (LDLs), for example, have become notorious as cholesterol "bad guys" because high concentrations predispose to heart attacks. Within the past few years, however, a variety of studies have indicated that high concentrations of lipoprotein(a) are an independent risk factor for heart attacks. Lipoprotein(a) concentrations are genetically determined and apparently are not reduced by diet or by lowering the cholesterol and fat contents of the diet as LDL concentrations may be.

Neither the normal role of lipoprotein(a) nor its contribution to heart attack risk is well understood at this time. The resemblance of the apo(a) sequence to that of plasminogen points in a possible direction, however.

Plasminogen is the inactive precursor of plasmin, a protein-splitting enzyme that helps to dissolve blood clots by digesting fibrin, the principal protein of clots. The apo(a) protein is not itself a protein-splitting enzyme, but its structural similarity to plasminogen raises the possibility that it could interfere with plasminogen or plasmin action, thereby contributing to blood clot formation.

Apo(a) might, for example, bind to the fibrin in clots and protect it from being dissolved by plasmin. Because lipoprotein(a) is cholesterol-laden, this could also foster atherosclerotic plaque formation at the sites of blood vessel damage.

There is currently some disagreement, however, about whether apo(a) binds to fibrin or interferes with clot dissolution. At the meeting, Lawn reported that his group finds little indication that it does either of these things. But evidence from other groups suggests that fibrin binding and prevention of clot dissolution may be important aspects of apo(a) action, and the issue remains to be resolved.

Another possibility now being explored is that apo(a) may bind to the plasminogen receptors located on the inner vessel walls and prevent plasminogen from binding to them, a step that is necessary for its activation. The growing recognition of the importance of lipoprotein(a) as a risk factor for heart attacks will continue to provide an impetus to efforts to find out what it does.

Blood Clotting May Increase with Age

The incidence of coronary heart disease and other clotting disorders goes up as people age. Data presented at the NHLBI symposium by Robert Rosenberg of the Massachusetts Institute of Technology raised the possibility that an age-related increase in the blood's tendency to clot may contribute to the increase in clotting problems.

Clot formation requires the activity of a cascade of enzymes that act in sequence, ultimately producing thrombin, itself an enzyme that carries out the final step in clot formation. "The balance between thrombin formation and thrombin inhibition determines whether clotting is normal or whether the blood vessels fill with clots," Rosenberg says.

Investigators from several laboratories, including Rosenberg's, have identified two separate systems that act to inhibit thrombin production or action in blood vessels. In one, a protein called antithrombin, in conjunction with the natural anticoagulant heparin, complexes directly with thrombin to prevent it from working.

The second shuts down thrombin synthesis when its concentration begins to build up. The thrombin binds to thrombomodulin, a receptor on the inner blood vessel walls, and consequently develops a greatly enhanced ability to act on a blood protein called protein C. This reaction generates a product that in turn attacks and inactivates components of the enzyme cascade that produces thrombin.

If either of these anticlotting systems fails to operate as it should, the risk of developing abnormal blood clots increases. Persons who have hereditary deficiencies of antithrombin or protein C, for example, often get clots in the lungs or the deep leg veins.

Moreover, most heart attacks are caused by blood clots that form in the coronary arteries, usually at sites that have already been narrowed by atherosclerosis, and cut off the blood supply to the heart muscle. Why such clots occur is not fully understood, although a deficiency in the anticlotting mechanisms may contribute.

In any event, the Rosenberg group has biochemical evidence that thrombin activation goes up as people age. The increase may be due to defects in anticoagulation, although apparently not to declines in antithrombin or protein C concentrations. There are, however, other possible ways in which the clotting controls might become impaired, and the Rosenberg group is now looking into them.

■ JEAN L. MARX