ARTERY CLOGGING AND APO-B

Some people seemingly at low risk for cardiovascular disease defy medical wisdom and have a heart attack or stroke. The explanation may lie in apolipoprotein B-100, a large protein made in the liver.

By JOANNE SILBERNER

First it was cholesterol as the evildoer in cardiovascular disease. The waxy molecule received primary blame as the cause of hardening of the arteries, which can cause strokes and heart attacks.

As researchers took a closer look at the mechanism of atherosclerosis, they found that it was a cholesterol-carrying particle containing low-density lipoprotein (LDL), as opposed to cholesterol carried by high-density lipoprotein (HDL), that was the source of the problem. Today measurement of relative HDL and LDL levels has become routine for many doctors evaluating their patients' risk of heart disease or stroke.

But the real villain may turn out to be LDL's sole protein component, a molecule called apolipoprotein B-100 (apo-B). It is fast becoming the focus of cardiology researchers, who think they could have a line on a previously unknown cause of atherosclerosis. "Apo-B," says one, "is probably the most crucial protein involved in atherosclerosis."

Variations in the structure or concentration of apo-B, they say, may explain why some people with normal cholesterol and LDL levels nevertheless get atherosclerosis and die of heart attacks. A 1980 study of 100 people being examined for hardening of the arteries showed that apo-B was a better predictor of actual atherosclerosis than were cholesterol or LDL levels.

Though the frequency of high or aberrant apolipoprotein-B isn't known at the moment, preliminary studies of the genetics of the molecule indicate that individuals with particular forms or high levels of apo-B may face a greater risk of heart disease. Finding a way to identify people in this group would enable them to lower their odds of eventual heart disease by modifying their diets or using cholesterol-lowering drugs at an early age.

The attention being paid to apo-B results from an understanding of how its carrier, LDL-cholesterol, causes problems. LDL's action was worked out in the 1970s by Joseph Goldstein and Michael Brown, two University of Texas Health Science Center at Dallas researchers who won the 1985 Nobel prize in medicine for their efforts (SN:10/19/85,p.246).

They determined that cells in the body contain LDL receptors on their surfaces that enable them to pull in LDL-cholesterol particles from the bloodstream. Other laboratories later discovered that the apo-B protein is key to the process; it is the part of the LDL-cholesterol particle that latches onto the receptor. Subsequent "ingestion" of the particle supplies the cell with the cholesterol it needs for the manufacture and repair of cell membranes, and in some cases for the synthesis of certain hormones. The action also removes LDL-cholesterol from the blood, eliminating it from potential involvement in atherosclerosis.

While most people have two normal genes for the LDL receptor per cell, one in 500 people have only one adequate gene per cell. With fewer functioning LDL receptors on the cells, high levels of LDL-cholesterol remain in the blood, hastening or causing atherosclerosis. People with this condition have twice the normal blood LDL level, even at birth. By age 35, they may have a heart attack; Brown and Goldstein estimate that up to 85 percent of men with familial hypercholesterolemia will have a heart attack before they reach 60.

A second, much smaller group has no normal genes for LDL receptors. This condition, which afflicts about one in a million people, can cause heart attacks in children as young as 2 years old.

But while approximately half of all deaths in the United States are caused by atherosclerosis, aberrant LDL receptors don't account for all or even most of them — fewer than 5 percent of heart attacks in people under the age of 50, for example, are due to LDL receptor problems.

Apo-B researchers say that the other part of the cell surface interaction — the apo-B protein on the LDL particle, rather than its receptor on the cell — may explain at least some of the rest.

After studies showed a link between high levels of apo-B and coronary artery disease, researchers began investigating the protein structure and function. The amino acid building blocks of the protein have now been sequenced, and certain forms of the molecule have been associated with heart attacks.

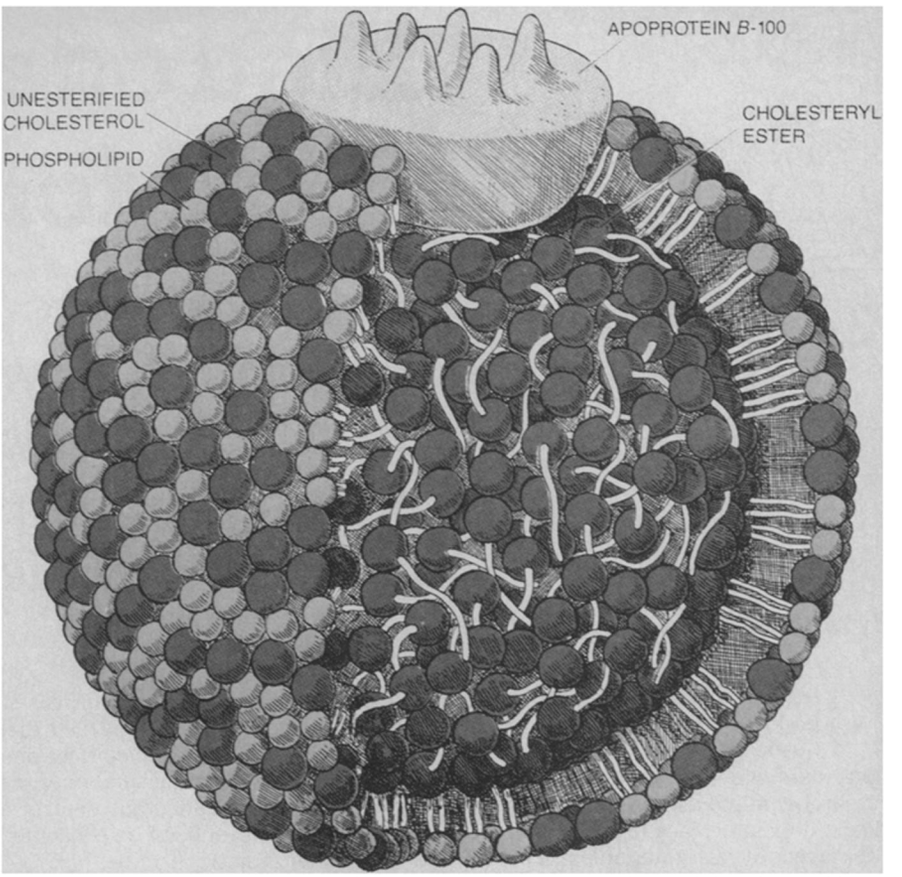
The sequencing was reported by U.S., Belgian, English and Canadian laboratories last Oct. 23 in two papers in NATURE. At more than 4,500 amino acids, it is the largest protein ever sequenced. Now that the protein has been characterized, says Antonio M. Gotto Jr. of the Methodist Hospital in Houston, one of the people who sequenced it, differences among people can be analyzed to see how amino acid changes relate to heart disease.

Even without the exact protein sequence, Robert A. Hegele and Jan L. Breslow of New York's Rockefeller University and their colleagues there and at several other institutions have been able to associate particular forms of the apo-B gene with heightened risk of heart attacks. To study the gene, they isolated DNA from 84 people who had had heart attacks and 84 people of the same age, sex and neighborhood who had not had heart attacks, and cut the DNA with enzymes that slice it at specific locations. To the fragments they added probes that recognize segments of the apo-B gene and then checked the length of the apo-B gene fragments. When the apo-B genes in different people are identical, they yield the same fragment pattern; genetic variations result in different fragment lengths.

Using two enzymes, the researchers identified seven fragment patterns; three of the seven correlated with coronary heart disease, independent of the total level of apo-B or LDL.

In a "backwards" look at the predictive value of the genetic differences, Hegele and his group used the genetic variables alone to predict 76 percent of the heart attacks, whereas currently used clinical and biochemical variables predicted 63 percent. With the use of different DNA-cutting enzymes, the predictive power could be improved, says Hegele.

The study, which was presented at the recent American Heart Association meeting and in the Dec. 11, 1986, NEW ENGLAND JOURNAL OF MEDICINE, was not designed to determine whether the genetic variations found actually cause heart disease. It did show that they may play a role, but measurement of the fragment lengths is not yet ready for widespread use, says Hegele. The disease-linked genetic patterns they found are relevant only for the test group, Caucasians, and the study needs to be replicated for this and other groups, he says.



Brown, Goldstein, SCI. AM. 11/84 © 1984 W.H. Freeman & Co.

Apo-B is the protein component of the low-density lipoprotein particle, pictured here. Low-density lipoprotein is the major cholesterol carrier in the blood. High levels or particular forms of apo-B may increase a person's risk of heart disease.

High levels of the protein are already being used to predict heart disease risk – the tests have become common in Italy and France, says Antonio Gotto, and test kits are available in the United States. It may be useful in people from families in which heart disease is prevalent, but whose own cholesterol and LDL levels aren't particularly high. But measuring apo-B levels as a general screen for heart disease is premature, given the knowledge at hand, he says.

Eventually, says Gotto, it might be possible to screen people's genes early in life for the presence of risky apo-B genes. People who come up positive would be encouraged to alter their diet or take drugs that will alter their apo-B or cholesterol levels before atherosclerosis becomes established.

Therapeutically, antibodies to the protein could be used to clear LDL particles from the blood, and work has already begun in this area, says Gotto. Additionally, drugs that interfere with the protein's action can be sought.

As a research tool, apo-B could provide a quick test of the efficacy of drugs against heart disease – new drugs could be screened for their effects on apo-B production by cultured cells, with drugs that decrease apo-B secretion selected for further trials.

Further investigation of the protein may also clear up a cardiology mystery – how some people with all the customary risk factors manage to avoid heart disease, while others whose arteries should be clean die of heart attacks, their arteries clogged with cholesterol deposits.

"The classic example was Winston Churchill," says Hegele. "He had a very stressful life, was very sedentary and was overweight, yet he lived to be 92. And somebody like Jim Fixx, the runner, died at 52 of a heart attack and he had a very prudent diet and was in good shape.

"There are differences in people that go beyond lifestyle, beyond smoking and diet and exercise," he says, and differences in the apo-B genes are one possible explanation.

As the sole protein component of the "bad" LDL, says James Scott of the Clinical Research Centre in Harrow, England, who was also involved in the sequencing of apo-B, "it's a very important candidate for genetic action." Abnormalities, he says, could occur in the transcription of the gene into protein, or in the nature of the protein product.

"What will come out of this research," says Scott, "is a much more clear understanding of the molecular level of the problem involved."

Given the association between apo-B levels and structure and heart disease, is apo-B analysis going to become a routine clinical test?

"It's too early to tell," says Gotto. "We're still at the discovery area at the present time. Until we know how the changes relate to atherosclerosis, they should remain as a research tool rather than something done in clinical labs." Says Scott, "It's a complex, long-term process but the answers are coming fast." □