Atherosclerotic Plaques: Competing Theories Guide Research

Atherosclerosis is often frustrating to study and treat. The disease has no symptoms in its early stages, and since there is not yet an adequate noninvasive way to detect this disease, people generally do not know that they have it until they suffer a heart attack or stroke. By that time, no one can tell the victims why they, in particular, developed atherosclerosis, or what they could have done to prevent it.

A major problem in understanding the etiology of atherosclerosis is that, like cancer, it seems to be a disease of many causes. Cigarette smoking, high blood pressure, high concentrations of blood lipids and cholesterol, excessive intake of animal proteins, various genetic disorders, and numerous other factors have all been linked to the development of this disease in susceptible individuals. This multiplicity of possible causes has stimulated investigators to look for some sort of common denominator—initial events in the genesis of atherosclerotic plaques that can be set in motion by any of these agents. The hope is that an understanding of how atherosclerotic plaques form will lead to new ways to prevent or reverse their development.

This is the second in a series of Research News articles examining recent developments in research on heart disease.

From examinations of human atherosclerotic plaques and from studies of plaques in animals, researchers have come to agree that plaques consist primarily of arterial smooth muscle cells. These cells presumably proliferate and migrate from the middle layer to the inner layer of the arterial wall; as the plaques form, various substances such as connective tissue proteins and lipids accumulate. Since the proliferation of smooth muscle cells seems to be central to the formation of plaques, investigators believe that an understanding of why and how plaques originate will come when they can determine what causes arterial smooth muscle cells to proliferate. To this end, they are, for the most part, examining the ramifications of two hypotheses.

The first hypothesis is an old one whose recent widespread support is based mainly on studies of lesions of animal arteries. It states that plaques form in response to frequently recurring injuries to arterial walls. The competing hypothesis, which was first proposed about 3 years ago, states that plaques are benign tumors, each of which is formed by progeny of a single cell that has lost control of its growth. The two hypotheses lead to different predictions of how atherosclerosis can be prevented or controlled.

Proponents of the response-to-injury hypothesis point out that almost any kind of chronic damage to animal arteries leads to the development of lesions resembling human atherosclerotic plaques. For example, Sean Moore and his associates at McMaster University in Canada mechanically injure rabbit arteries with catheters. C. Richard Minick of Cornell University injures rabbit arteries with antigen-antibody complexes. Lawrence Harker and Russell Ross of the University of Washington School of Medicine injure baboon arteries with the amino acid homocysteine, and Ross and his associates injure monkey arteries by raising the concentration of lipids in their blood to an equivalent of that of people with hypercholesterolemia. (The way in which high concentrations of lipids injure monkey arteries is not known, but it can be shown that injury does occur.) Supporters of this hypothesis suggest that humans can suffer arterial injuries from high blood pressure, antibodies to or carbon monoxide from cigarette smoke, high concentrations of blood lipids and cholesterol, and numerous other conditions.

Events in Plaque Development

By observing the genesis of lesions produced by injuries to arterial walls of animals, investigators have begun to piece together a sequence of events that is common to lesion formation in all animal species studied. They propose and have some evidence that the same sequence of events occurs when humans develop atherosclerotic plaques.

The first event in the development of lesions in animals is injury to the arterial endothelium, which is a thin layer of cells that coats the inner walls of arteries and controls the passage of large molecules in the blood through to the inner layers of the arterial wall. Once this barrier is disrupted, substances that normally are screened out by the endothelium can reach smooth muscle cells. For example, plasma lipoproteins, which are believed to supply lipids to the developing plaques, can then more easily contact smooth muscle cells. At the same time platelets adhere to the newly exposed tissue, aggregate, and release substances that may stimulate cell proliferation. Subsequently, smooth muscle cells, which are normally present mainly in the middle layer of the artery, begin to proliferate and migrate to the inner layer where the injury occurred.

The increased number of smooth muscle cells are surrounded by large quantities of connective tissue proteins and other macromolecules. As Ross and his associates have shown, arterial smooth muscle cells secrete these substances both in vivo and in vitro. Their accumulation is presumed to occur concomitantly with the multiplication of the smooth muscle cells. Supporters of the response-to-injury hypothesis claim that the resulting lesions in animals appear to be identical to lesions in humans that are considered to be precursors of atherosclerotic plaques.

Several groups of investigators, including Moore, Ross, and their associates, found that smooth muscle cells of injured animal arteries continue to proliferate and accumulate connective tissue and lipids if the injuries are sustained or repeated. The lesions then resemble advanced atherosclerotic plaques in humans. Lesions in animals regress and eventually disappear if the injuries are not repeated, which leads many researchers to believe that repeated injury may be necessary for atherosclerosis to develop.

The central question that arises from this research is, Why do smooth muscle cells proliferate when the arterial endothelium is injured? In recent years, it has been suggested that the answer may lie in the responses of smooth muscle cells to various substances in the blood. One such substance seems to be present in the blood of people or animals with elevated concentrations of blood lipids. Robert Wissler, Katti Fischer-Dzoga, and their colleagues at the University of Chicago find that low-density lipoproteins (which carry cholesterol) from the blood of monkeys with hypercholesterolemia cause arterial smooth muscle cells to proliferate in tissue culture. No other fraction of serum from these monkeys produces this effect; neither do the low-density lipoproteins from

monkeys with normal serum cholesterol concentrations produce this effect.

Other substances in the blood that may cause smooth muscle cells to proliferate are those released from platelets that aggregate at the site of injury to arterial walls. Ross and his colleagues report that a growth stimulating factor is released from platelets when blood clots. When quiescent smooth muscle cells from monkey arteries are grown in tissue culture, this factor causes them to divide. When these cells are grown in the presence of cell-free serum, the cells remain in a resting state and maintain their original density. The addition of a protein released from platelets stimulates these cells to synthesize DNA and divide. R. Bruce Rutherford of the University of Washington School of Medicine and Ross find that within 48 to 60 hours after they add the platelet protein, the number of cells doubles. These investigators point out that arterial smooth muscle cells are normally exposed only to filtrates of plasma, since the endothelium prevents the entry of platelets and other constituents of whole blood.

Plaque Formation Is Prevented

If a factor released from platelets is necessary in order for lesions to form, it should be possible to prevent the lesions that follow endothelial injury by preventing platelets from releasing this factor. Recently, several groups of investigators demonstrated that this can be done.

Moore, Robert Friedman of McMaster University, and their associates injured rabbit aortas by placing catheters so that they repeatedly hit the arterial walls. This injury causes fibrous plaques to develop. When the investigators treated the animals with antibodies to rabbit platelets, the lesions did not form. Similarly, Michael Stemerman, of Beth Israel Hospital in Boston, and his associates injured rabbit arteries with a catheter to which a balloon was attached. They insert the catheter, inflate the balloon, and rub off endothelial cells with the balloon. When they do so, plaques normally occur at the sites where the cells are rubbed off. After these investigators destroy most of the rabbits' platelets with antibodies, they find that plaques no longer form in response to the injury.

A different kind of injury was studied and its atherogenic effects prevented by Harker, Ross, and their associates. They continuously infused homocysteine into the bloodstreams of baboons for 3 months. Harker and Ross found that the animals lost 10 percent of their arterial endothelial cells and developed lesions. Another group of baboons was given both homocysteine and the drug dipyridamole, which inhibits platelet functions and prevents the release of factors from platelets. These animals lost endothelial cells but did not develop lesions.

Platelets may be associated with the genesis of human atherosclerotic plaques as well as plaques in animals. The admittedly preliminary evidence is based on measurements in humans of platelet survival times, which indicate how long platelets circulate in the blood before breaking down. Platelet survival times are expected to decrease when platelets accumulate and break down at the site of arterial injury. For example, Harker, Ross, and their associates reported that platelet survival times are decreased 50 percent in baboons infused with homocysteine.

In addition to studying baboons, Ross, Harker, and their associates measured platelet survival times of patients with homocysteinurea—an inborn error of metabolism that causes people to have large amounts of homocysteine in their plasma. These people typically die of atherosclerosis before they reach 30 years of age. The investigators found that a group of patients with this disease had platelet survival times that were 50 percent lower than those of controls.

Ross, Harker, and their associates further found that they were able to treat their homocysteinuric patients so as to increase their platelet survival times. Some of the patients responded to the vitamin pyridoxine by producing less homocysteine, and they were treated with that vitamin. The rest were given dipyrimadole. All of the treated patients subsequently had normal platelet survival times.

The results of Harker and Ross were recently questioned by Joseph Schulman, S. Harvey Mudd, and their associates at the National Institutes of Health (NIH). They also measured platelet survival times in a group of patients with homocysteinurea and found them to be normal.

Mudd explains that the results of the study of the NIH group do not necessarily contradict those of Harker and Ross. The patients studied by the NIH group were less severely affected by homocysteinurea than the patients of Harker and Ross and presumably had less extensive arterial injuries. Measurements of platelet survival times are not particularly sensitive, and severe injuries to the arteries might be required before decreased platelet survival could be detected. It also remains possible (some say likely) that platelet factors are not solely responsible for the proliferation of smooth muscle cells and that normal platelet survival times do not necessarily indicate a lack of arterial damage and plaque formation. Conversely, shortened platelet survival times may not necessarily indicate that atherosclerotic lesions are progressing.

Stemerman and his associates recently obtained evidence that other factors may diminish the effects of platelet substances on the proliferation of smooth muscle cells in injured rabbit arteries. They find that after an artery is injured, smooth muscle cells of the resulting lesion begin to regress about 16 weeks after the initial injury. Yet these smooth muscle cells still have platelets attached to them. Healing begins when endothelial cells start to grow back over the lesions—a process that takes far longer than 16 weeks to be completed. Smooth muscle cells covered by new endothelial cells regress more rapidly than those not covered, according to Stemerman and his colleagues. Thus, the endothelium may be filtering out substances that stimulate, to some extent, the growth of smooth muscle cells. Stemerman points out, though, that the fact that smooth muscle cells regress while exposed to platelets indicates either that the cells can control their response to factors released from platelets or that other substances are involved in the stimulation of smooth muscle cell proliferation.

Lipoproteins and Plaque

One factor that may be necessary to sustain smooth muscle cell proliferation is an elevated concentration of low-density lipoproteins in the blood. Wissler and his associates, among others, find that, in monkeys and swine with hypercholesterolemia, advanced atherosclerotic plaques shrink substantially when the animals' serum cholesterol concentrations are returned to normal. As the plaques shrink, the excess proliferation of arterial smooth muscle cells ceases. (The role of low-density lipoproteins and cholesterol in atherogenesis will be discussed in the next article in this series.)

Despite its widespread support among investigators, the response-to-injury hypothesis is based mainly on studies of animals. A major obstacle to extending findings from animal studies to humans is that initial events in human atherosclerosis are nearly impossible to identify. Human lesions are generally seen only at death or on the removal of an artery, and in neither case can the temporal development of the lesions be followed. Some investigators object to the

response-to-injury hypothesis by saying that lesions of injured animal arteries are not necessarily comparable to human atherosclerotic plaques. There is disagreement about the validity of this objection among investigators. Proponents of the hypothesis that plaques are benign tumors—the monoclonal hypothesis—avoid this criticism since evidence supporting this hypothesis comes from studies of human plaques.

Earl Benditt and John Benditt of the University of Washington School of Medicine advanced the monoclonal hypothesis on the basis of an analysis of human plaques obtained at autopsies. Their analysis relies on a method that had been used previously to support the contention that benign uterine tumors made up of smooth muscle cells are derived from single cells. This method is based on the generally accepted beliefs that only one of the two X chromosomes in a given cell of a female expresses its genes and that which X chromosome in a cell is active is decided at random during embryo development. All progeny of a particular cell express genes from the same X chromosome as their parent, but neighboring cells are often derived from different parent cells and thus often express genes from different X chromosomes.

One particular gene carried on X chromosomes codes for the enzyme glucose-6-phosphate dehydrogenase (G6PD). This enzyme can occur in two distinguishable forms, and black females tend to be heterozygous for the gene. Thus cells from black females who are heterozygotes will synthesize, at random, one or the other form of G6PD.

The Benditts examined atherosclerotic plaques from four black females and found that most cells collected from a single plaque expressed one or the other form of G6PD, but not both. They interpret this to mean that each plaque was generated by progeny of a single cell. In contrast, they found that cells from samples of artery walls adjacent to the plaques tended to produce both forms of G6PD.

In the 3 years since the Benditts advanced the monoclonal hypothesis, their results have been confirmed by other groups of researchers. Various investigators have published speculations as to how this hypothesis could be further tested and how previous results could be interpreted in light of it. Wissler, for example, believes that investigators should address the question of whether the arterial cells that seem to proliferate so readily when exposed to plasma lipoproteins are transformed cells. Ross points out that transformed arterial smooth muscle cells may not be affected by the same growth stimulants, such as factors released from platelets, as are normal arterial smooth muscle cells grown in tissue culture. Thus, in vitro studies of growth stimulants of smooth muscle cells may have to be reexamined.

Earl Benditt suggests that the meaning of proposed risk factors for atherosclerosis should be assessed in light of the monoclonal hypothesis. For example, cigarette smoking may be associated with this disease because cigarette smoke contains mutagens. And plasma lipoproteins may carry fat-soluble mutagens to arteries where these mutagens may pass through the arterial endothelium and contact smooth muscle cells. Benditt points out that diets high in fat have been associated with the development of various cancers, such as breast cancer, as well as atherosclerosis. He proposes that such diets may cause both cancers and plaques by similar mechanisms.

Although other investigators have confirmed the Benditts' results, several groups have recently raised objections to their interpretation. Philip Fialkow of the University of Washington, for example, points out that there is some evidence that plaques develop in layers. A group of cells may proliferate, then most die, and a few remaining cells proliferate again. If this is the case, a plaque could end up with cells of a single enzyme phenotype even though the plaque originated from many cells. Similarly, George Martin and his associates at the University of Washington School of Medicine argue that the Benditts' data do not necessarily indicate that plaques are formed by mutated or transformed cells. After studying a variety of cell lines, they discovered that cells that divide rapidly enjoy a selective advantage. Thus progeny of a single cell might take over a plaque that had a multicellular origin.

Somewhat different evidence against the monoclonal hypothesis is reported by Wilbur Thomas and his associates at Albany Medical College. These investigators found that plaques in swine are not monoclonal. They radioactively labelled the normal arterial tissue and induced lesions by feeding the animals diets high in cholesterol. If each lesion were formed from a single cell, the radioactivity of each lesion should be substantially less than the radioactivity of the surrounding cells of the artery. This did not occur. Instead the radioactivity of each lesion was not sufficiently diluted for it to be derived from one rather than many cells.

Thomas admits that the lesion in swine may not be analogous to those of humans, but still maintains that the evidence advanced by the Benditts is not sufficient to support the monoclonal hypothesis. Despite these arguments against the monoclonal hypothesis, no one has yet succeeded in ruling it out. It, like the response-to-injury hypothesis, continues to have both supporters and detractors. Both hypotheses continue to suggest new experiments whose results, many believe, are narrowing the range of possible causes of and ways to prevent atherosclerosis.—GINA BARI KOLATA

The 1976 Nobel Prize in Economics

The Nobel Prize in Economics for 1976 has been awarded to Milton Friedman of the University of Chicago. The weight and range of his scholarly contributions fully justifies this honor, which has long been overdue. Friedman has advanced many original and fruitful ideas bearing on a wide range of problems and phenomena. His ideas influenced many questions asked, the nature, of the theoretical analysis developed, and the empirical examinations applied by the profession.

The recipient's scholarly interests have dealt with price theory (analysis of demand and household behavior) and utility theory (behavior under risk), and have covered important problems in macrotheory (the consumption function). Moreover, Friedman's work has reshaped monetary theory and influenced monetary policy, has produced critiques of the profession's standard arguments on evaluation of hypotheses, and has pioneered important ideas in statistical analysis and the application of capital theory to human knowledge and skills. Perhaps most importantly, Friedman pointed the way to imaginative extensions and applications of economic