Infections: A Cause of Artery-Clogging Plaques?

SPECIAL FOCUS: CARDIOVASCULAR DISEASE

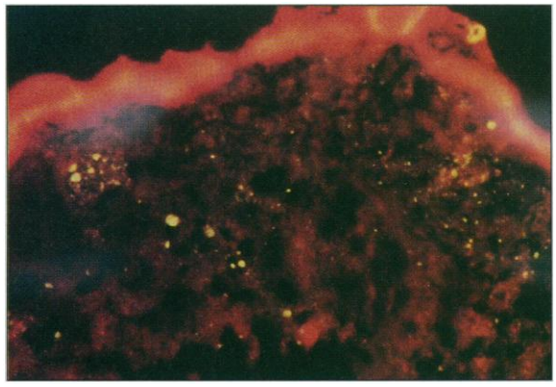
Recent evidence suggests that common bacteria and viruses contribute to the development of atherosclerosis, perhaps by triggering inflammation

Some cardiovascular disease experts are finding inspiration in ulcers. Until a few years ago, most experts attributed stomach ulcers to factors much like those thought to cause the fatty arterial deposits, or plaques, that trigger heart attacks and many strokes: diet, lifestyle, and an individual's own genetic susceptibility. Then researchers discovered that many, if not most, peptic ulcers are caused by a common bacterium, *Helicobacter pylori*—a finding that opened the way to treating ulcers with antibiotics. Now, cardiovascular researchers are being tantalized by hints that the bacteria and viruses that cause such common ailments as pneumonia, gum disease, and, yes, ulcers could be at least contributing factors in plaque formation.

So far, the evidence that infections play such a role is largely circumstantial. Researchers have found signs of infection, such as antibodies to certain pathogens, more often in heart disease patients than in healthy individuals. Investigators have also found microbial DNA, RNA, and proteins in the artery-clogging lesions. However, the actual organisms could not be extracted from plaques and successfully cultured, leaving open the question of how the infectious agents might contribute to formation of the plaques, if they contribute at all. "The organisms could be innocent bystanders," says Paul Ridker, a cardiologist at Brigham and Women's Hospital and Harvard Medical School in Boston. "These data are helpful but hardly definitive."

Still, everyone agrees that the idea that infections might promote atherosclerosis, perhaps by triggering inflammation of the vessel walls, should not be dismissed. "The evidence that bacteria are found in plaques needs to be taken seriously," says Peter Libby, chief of the cardiovascular division at Brigham and Women's and Harvard Medical School, who helped put together a special panel of experts to sift through the current data and look for answers. The panel, commissioned by the National Heart, Lung, and Blood Institute in Bethesda, Maryland, concluded that the current data were "intriguing" and called for further studies of the infection-atherosclerosis link.

If bacteria do turn out to trigger blood vessel disease, then relatively inexpensive antibiotic regimens might be added to the current cholesterol-lowering, blood pressure–reducing heart disease prevention repertoire. It's still far too early to recommend that heart patients generally be put on antibiotics, with their potential for fostering resistant strains of bacteria. But at least half a dozen studies are already under way to test whether these drugs can prevent heart attacks or other coronary events in patients with atherosclerosis and other heart conditions. "At some point, you have to study the organisms when and where the disease is actually taking place," says Michael Dunne, senior director of clinical research at Pfizer in Groton, Connecticut, a pharmaceutical giant that is conducting one of the antibiotic trials.



B. MUHLESTEIN

Suspicious tracks. Fluorescent staining (yellow) shows large numbers of *Chlamydia* bacteria in this coronary artery plaque.

The current interest in a possible link between infection and cardiovascular disease had its roots in several papers published in the late 1980s. In one, for example, Petra Saikku and his colleagues at the University of Helsinki in Finland found that 27 out of 40 heart attack patients and 15 out of 30 men with heart disease carried antibodies related to a bacterium called *Chlamydia pneumoniae*, which is known primarily as a cause of sexually transmitted diseases. Compared to the seven out of 41 control patients who had such antibodies, the results were significant, even though the overall study was small. In a similar vein, Joseph Melnick's group at Baylor College of Medicine in Houston, Texas, and others found that 70% of patients undergoing surgery for atherosclerosis carry antibodies to cytomegalovirus (CMV), a common virus that causes respiratory infections, while only 43% of normal controls do.

At the time, though, many physicians argued that even if the patients did have more infections than healthy controls did, that might be a simple coincidence. They noted, for example, that people who have such infections are more likely to smoke, be older, have poorer access to health care, and live in poverty than those who are healthy. "Those are the same kind of people who have heart attacks," says Ridker. "I think you have to be careful when interpreting the data." Dunne agrees: "You could argue that these [studies] were not the strongest link." But he adds, "At least they were something that put a bug and heart disease on the same radar map."

Heart experts began taking the possibility of such a link more seriously in the early 1990s, when the pathogens started turning up in the plaques themselves. Techniques including the polymerase chain reaction, which amplifies trace nucleic acid sequences, and immunohistochemistry, which uses specific fluorescent probes to light up telltale microbial proteins, enabled researchers to track down the organisms. "I looked at those data and thought, 'Wow, here we might have the smoking gun: bacteria in a plaque,' " recalls Brent Muhlestein, a cardiologist at the LDS Hospital and the University of Utah in Salt Lake City. "I had always gone on the assumption that the arterial plaques were sterile."

Indeed, research by Muhlestein and his colleagues later turned up *Chlamydia* proteins in 79% of plaque specimens taken from the coronary arteries of 90 heart disease patients. The protein could be detected in fewer than 4% of heart artery walls of normal individuals. Animal studies then provided more direct evidence that the bacterium might contribute to plaque formation. The Muhlestein group and others showed that infecting rabbits with *Chlamydia* measurably thickens the arterial walls of the animals, especially those given high-fat diets or those genetically predisposed to high blood cholesterol.

What's more, Muhlestein's team also gave one subset of infected rabbits doses of azithromycin, an antibiotic used to treat *Chlamydia* infections, and found that the arteries of the treated animals looked more like those of uninfected rabbits. "We concluded that infection with *Chlamydia* actually accelerates the development of atherosclerosis, and treatment with azithromycin prevents it, in the rabbit model," Muhlestein says.

Since *Chlamydia* and CMV were implicated as possible contributors to heart disease, other microbes have joined them as suspects. Several teams have evidence implicating the bacteria that cause gum diseases such as gingivitis as a factor in atherosclerosis. In a study reported 2 years ago, for example, James Beck's group at the University of North Carolina, Chapel Hill, looked at dental data collected on 1147 men between 1968 and 1971 and found that those with dental infections tended to have a higher risk of heart disease and strokes. And in the May issue of the journal *Circulation*, Vincenzo Pasceri's group at the Universitá Cattolica del Sacro Cuore in Rome, Italy, described the results of a small-scale epidemiological study suggesting that *H. pylori* might be involved in heart disease.

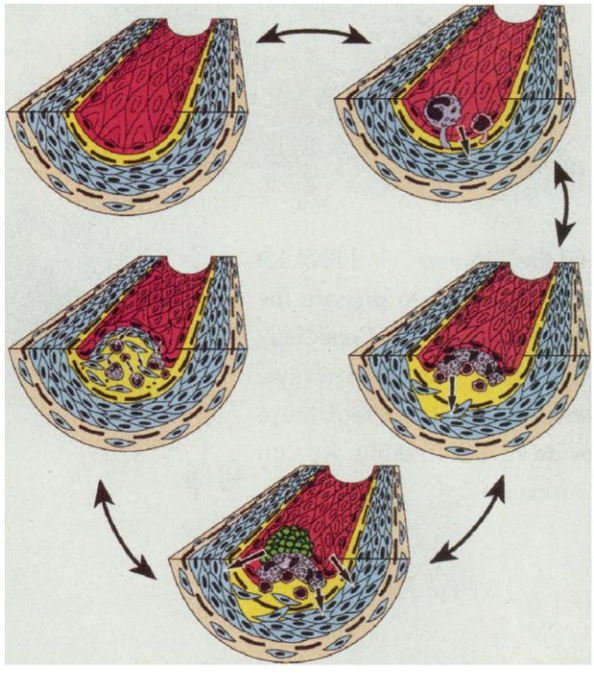
The results of these studies are far from the final word, especially because they are plagued by the same confounding factors as the *Chlamydia* and CMV studies: Those with bad teeth or ulcers tend to be older, to smoke, and to live in poverty. In addition, no one knows how infections might foster plaque development, and the puzzle is deepened by the range of pathogens under suspicion. Researchers are searching for a common denominator that might link them. One popular hypothesis is that inflammation triggered by the infectious organisms might be a key, as at least a decade of research has already implicated inflammation as contributing to plaque formation.

Evidence that infections might be working that way comes from Pasceri's group in Rome. Their *Helicobacter* study showed that only the more virulent of two inflammatory *Helicobacter* strains seemed to correlate with increased incidence of heart disease. Although 48% of heart disease patients carried the more aggressive organism, only 17% of controls did. By contrast, researchers found similar blood levels of antibodies to the more docile *Helicobacter* strain in both groups of patients.

But it's also unclear how microbes might work in arteries that are some distance from their primary infection sites. Brigham and Women's Libby suggests that there may be what he calls an "echo effect," in which infections cause a response not only at the site of infection, say, the intestine, but also at secondary sites such as the artery wall. He suggests, for example, that the organisms release toxins into the bloodstream or display molecules on their surfaces that exacerbate inflammatory reactions at the blood vessel linings. Infection echoes could add to other risk factors such as smoking to foster plaque growth.

But even Libby cautions that his echo theory is indeed that: a theory. More definitive answers about whether there is any link between microbes and cardiovascular disease are needed. Those could come from clinical trials designed to test whether antibiotics can prevent the disease.

The results reported publicly so far have been inconclusive, however. For example, this March at the American College of Cardiology meeting in Atlanta, Muhlestein described preliminary results from a trial of the antibiotic azithromycin, which he and his colleagues are conducting in 300 patients with previous heart problems. The antibiotic did not reduce heart attacks or other clinical events such as severe chest pain after 6 months, he said. But it did reduce the blood levels of several inflammatory molecules, and Muhlestein asserts that 6 months might be too soon for an effect to show up in the relatively small number of patients. "We'll be following up the patients for 2 more years to see if there is a reduction in heart attacks, strokes, and other clinical events," Muhlestein says.



R. ROSS, *NATURE* 362, 801 (1993)

Crime scene. Plaque formation may be initiated when an injury allows inflammatory cells like macrophages and lymphocytes to penetrate the arterial lining (upper right). Those cells, in conjunction with smooth-muscle cells (blue), platelets (green), and possibly infectious microbes, produce cytokines and other growth-regulatory molecules that foster plaque growth.

It should be easier to see results in the ambitious Wizard trial, which is sponsored by Pfizer, the manufacturer of azithromycin. Having just completed patient enrollment, Wizard has gathered 3500 heart disease patients from medical centers in the United States, Canada, and Europe who all have documented atherosclerosis and have tested positive for *Chlamydia* antibodies. The plan is to give half the participants the antibiotic and the other half a placebo. The patients will then be followed for at least 3 years to observe whether the drugs decrease the incidence of heart attack or other coronary events.

"This is kind of a risky trial," cautions Pfizer's Dunne. He notes, for example, that no one knows the best time to give the drug—it might be too late after heart disease has already developed—nor does anyone know what's the best drug dosage to use.

But given the rewards that would come if a trial turns up positive, these uncertainties have not daunted other drug companies that make antibiotics for treating *Chlamydia* infections. Abbott Laboratories in North Chicago, Illinois, is currently testing their drug, called clarithromycin, in heart patients in Europe; Hoechst Marion Roussel in Kansas City, Missouri, has completed a 270-patient pilot study with a compound called roxithromycin and is continuing with further studies; and the National Institutes of Health is funding a study of azithromycin, to be conducted on 3500 patients through the University of Washington, Seattle. "If any one of these trials turns out to be positive, I am sure there will be a whole family of studies that will start," Dunne predicts. "We've got to start somewhere, and this is a question well worth answering." **–TRISHA GURA**

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ADDITIONAL READING

P. Libby *et al.*, "Roles of infectious agents in atherosclerosis and restenosis: An assessment of the evidence and need for future research," *Circulation* **96**, 4095 (1997).

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A. Kol and P. Libby, "The mechanisms by which infectious agents may contribute to atherosclerosis and its clinical manifestations," *Trends in Cardiology and Medicine* **8**, 191 (1998).