A growing body of evidence suggests that a molecular marker for inflammation may be as crucial as cholesterol in assessing risk of heart attack

Does Inflammation Cut to The Heart of the Matter?

In the battle against heart disease, cholesterol has long held the title of molecular root of all evil. Now there's a competitor, or at least a co-conspirator.

The new molecule, known as C-reactive protein (CRP), is a marker of inflammation that has emerged alongside cholesterol deposition and clogged arteries as a significant factor in understanding heart disease. "What we've discovered is the commonality of inflammation and arteriosclerosis," says pathologist Michael Gimbrone of Harvard Medical School in Boston. "The very same cells and molecules that mediate inflammation and the response to pathogens and trauma are integral parts of the arteriosclerotic process." That discovery, the result of considerable work over the past decade, has spawned more questions, starting with the most fundamental one: Does the presence of inflammation trigger rising CRP levels that lead to heart disease, or is inflammation a byproduct of the disease? The answer could greatly affect the standard of care for the disease, from early diagnosis through treatment.

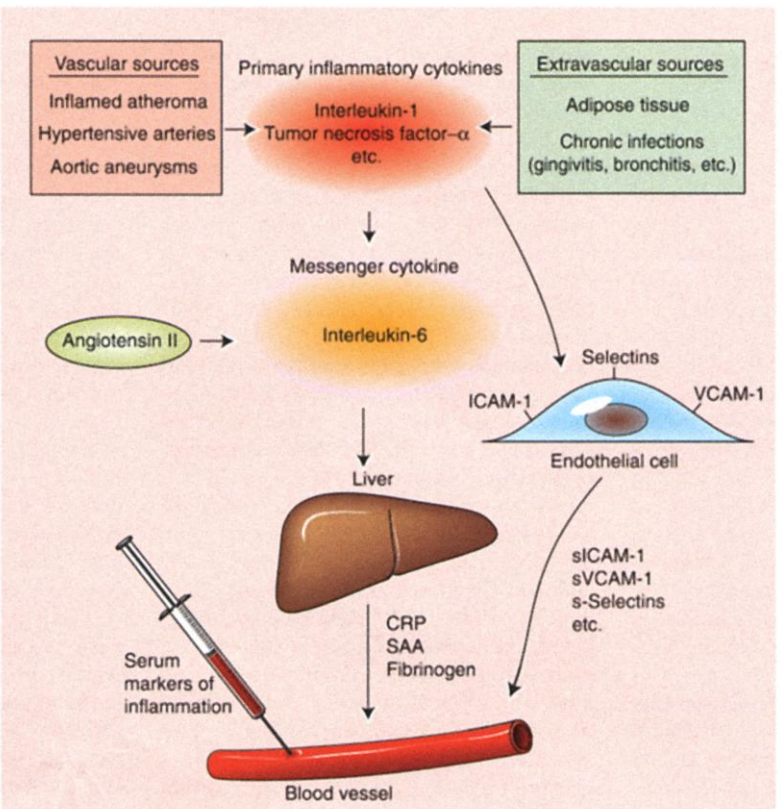
CRP was discovered in 1929 in the Rockefeller University laboratory of Oswald Avery. Barely measurable in the blood of healthy individuals, CRP is pumped out by the liver in much larger quantities during the early acute phases of disease or trauma. It "closely parallels the clinical course" of disease, Avery wrote in 1941. Since then, researchers have discovered that CRP is synthesized in the liver in response to interleukin-6, which is released by areas of inflammation. As a result, says Gimbrone, the amount of CRP can be considered "a total-body integrated readout of inflammation."

The role of inflammation in heart disease is only now becoming clear, as data accumulate suggesting that an overly aggressive response to inflammation is as critical to heart disease as high levels of serum cholesterol. This is a novel concept for a generation of biologists and cardiovascular disease specialists taught in their youth that arteriosclerosis was purely a process of lipid deposition. The more cholesterol in your blood, the argument went, the greater the blockage of your arteries, like sludge accumulating in a rusty pipe, and the greater the chance that a total block would lead to a heart attack. The arteries themselves played only a passive role.

"We thought about the process as a mechanical thing," says Harvard Medical School pathologist Nader Rifai. "But the puzzling thing was why some people who had significant blockage might never have a coronary event, whereas others with very little blockage would have a massive heart attack. Clearly there was more to the issue than just cholesterol blocking the blood flow." Indeed, high cholesterol levels alone could only predict at most half of all heart attacks. Now CRP and inflammation, say researchers, may be the variables that were missing in the heart disease–cholesterol equation.

The tide started to turn in the mid-1970s, at least in biology laboratories. Researchers, led by Gimbrone and his colleagues at Harvard, discovered how to culture and grow the endothelial cells that line the walls of arteries and veins. They spent a decade or more working out the molecular mechanism by which the endothelium could be activated in response to the kinds of molecules, known as cytokines, that normally induce an inflammatory response. These cytokines would activate genes on the endothelium that would initiate the first stages of arteriosclerosis. They also demonstrated that this process could be turned on by molecular risk factors for arteriosclerosis, such as cholesterol-carrying particles known as lipoproteins. Finally, and perhaps most important, says Harvard cardiologist Peter Libby, they demonstrated that endothelial cells not only respond to inflammatory molecules but generate their own such signals. "So, far from being innocent bystanders," he says, "the cells of the blood vessel wall could actually participate in the inflammatory response in an active sense."

By the early 1990s, Libby, Gimbrone, and others had assembled a picture of arteriosclerosis that involved molecules of inflammation,



Inflammatory statements. The complex interaction of inflammatory signals involves even endothelial cells as active participants in the inflammatory process.

ILLUSTRATION: P. LIBBY/HARVARD MEDICAL SCHOOL

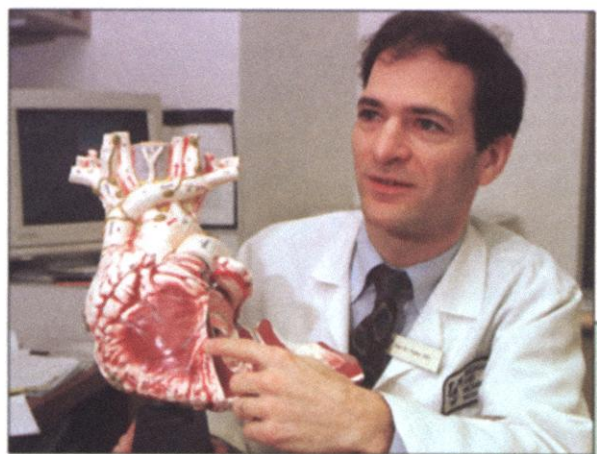
both cytokines and growth factors, from the moment the first oxidized low-density lipoprotein (LDL) particle adhered to the walls of the blood vessel until the moment, perhaps decades later, when the fibrous cap overlying an arteriosclerotic plaque burst open, causing a blood clot in the artery and a heart attack. "It's all a nice, neat little package," says Libby.

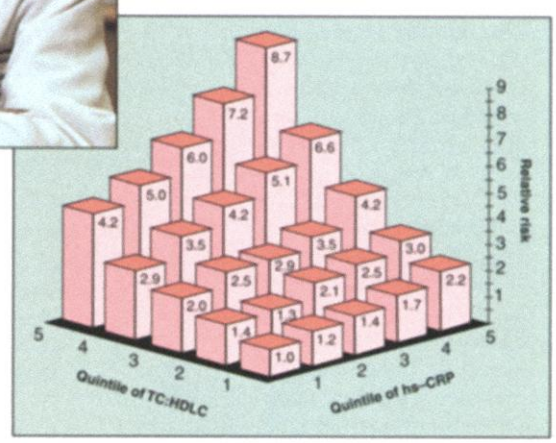
While vascular biologists were assembling the pieces of the inflammation-arteriosclerosis puzzle in the laboratory, CRP had developed into the subject of a routine medical assay in Europe and Japan. "It's used to detect the presence of inflammation or infection or to see if the patient is responding to treatment," says University College London biologist Mark Pepys. "It's a specific marker for infection and inflammation, but not specific to the extent that it will actually tell you what type of infection or inflammation the patient has."

Healthy individuals have only trace amounts of CRP in their blood: 90% have levels below 3 milligrams per liter. Once inflammation sets in, however, the levels can skyrocket 1000-fold or more. It's an "amazing response," says Pepys. "In the vast majority of diseases in which CRP goes up, the CRP measurement reflects very accurately—more so than any other objective measurement—how sick the patient is and how extensive the pathology."

In the early 1980s, researchers began to assess whether baseline CRP levels—those below, say, 10 mg/l, in apparently healthy individuals—might have predictive power. In particular, Pepys and Attilio Maseri, who is now at the University Vita-Salute in Milan, looked at CRP levels in patients admitted to a hospital with a heart attack and found that those with higher levels coming in had more dire outcomes. But the assays were insensitive and the research stalled for a decade, while cardiologists concentrated first on the role of cholesterol in heart disease and then on the role of hypercoagulation and thrombosis, or blood clots. Finally, in the early 1990s, Pepys and, independently, Russell Tracy and colleagues at the University of Vermont in Burlington developed CRP assays sensitive enough to measure accurately the very low CRP concentrations typical of healthy individuals. The research was primed to take off.

Researchers looked for correlations between a host of inflammatory markers and heart disease. CRP levels, they found, not only were an excellent predictor of heart disease but were better than any of the other inflammatory markers. CRP's major advantage over interleukin-6, cellular adhesion molecules, or tissue necrosis factor, for instance, as Harvard Medical School cardiologist and epidemiologist Paul Ridker quickly discovered, was that it had considerably more "clinical appeal." Ridker, for instance, found that virtually all inflammatory markers were elevated among individuals at risk of future heart attacks or strokes, but none of the other molecules were as biologically stable as CRP or had such a wide range of concentrations. Even among healthy individuals, the distribution of CRP levels in the blood spans a considerable range of values—from 0.01 mg/l up to 10 mg/l—making it relatively easy to compare low, medium, and high levels to disease outcomes. Barring a serious infection in the few weeks before the sample is taken, CRP levels also tend to be rock steady. They have neither diurnal nor seasonal variations, and they don't spike in response to food intake.





Independent predictors. Reflecting the underlying inflammatory response, CRP levels predict risk of future heart attacks and strokes among healthy men and women with low as well as high cholesterol levels, according to work by Paul Ridker (*above*) and colleagues.

Perhaps most important, CRP levels remain stable and measurable for decades in serum samples. Suddenly, says Pepys, "anyone who had conducted epidemiological studies over the years in cardiovascular disease—measuring cholesterol, clotting factors, and everything else—went back to their freezers and started pulling out samples by the thousands and measuring CRP."

The floodgates opened in 1996, with a report by Tracy, University of Pittsburgh epidemiologist Lew Kuller, and colleagues showing that baseline CRP levels in smokers with high cholesterol levels tracked with heart disease risk. Then Ridker and his colleagues showed that even in low-risk individuals, CRP levels acted as very potent predictors of risk of first-time heart attacks or strokes—and they did so even when cholesterol levels were low, which is the case in half of all heart attacks. "Suddenly we had an insight into why so many heart attacks and strokes were being missed by cholesterol screening," says Ridker. "Maybe inflammation, measured by this simple marker, was picking up a huge chunk of those we missed."

"The knock-your-socks-off thing about the observation," says Gimbrone, "is that you can go back and look at CRP in a serum sample from a decade earlier, and it will be predictive in an individual who yesterday had the heart attack." This was quickly confirmed by Pepys, Wolfgang Koenig, a cardiologist at Germany's University of Ulm, and collaborators, who reported a linear relation between CRP levels and heart disease in a randomly chosen sample of initially healthy middle-aged men.

Since 1997, the most systematic CRP work has been done by Ridker and his colleagues at Harvard. In a series of high-profile papers, they demonstrated that CRP levels also predicted heart disease risk in healthy women, that they were a more accurate predictor of risk than cholesterol levels, and that cholesterol levels and CRP levels were unrelated. This meant that CRP measurements could add to the predictive value of cholesterol measurements: Individuals with low cholesterol levels but relatively high concentrations of CRP—or vice versa—would still be at high risk of heart disease. Those with high levels of both CRP and cholesterol would be at the highest risk.

Ridker's group also looked at the effects on CRP levels and heart disease of aspirin, which is known to be an anti-inflammatory agent, and of statins, the cholesterol-lowering medications that significantly reduce heart disease risk. They showed that the magnitude of the heart disease benefits of a

CREDITS: (LEFT TO RIGHT) JON CHASE/HARVARD UNIVERSITY NEWS OFFICE; ADAPTED FROM P. RIDKER *ET AL.*, *CIRCULATION* **103**, 1813 (2001)

daily aspirin regimen was directly related to the CRP level: the higher the CRP levels, the greater the benefit of the aspirin. As for statins, their mode of action had always been mysterious, because their efficacy at reducing heart disease ran ahead of their known effect on cholesterol. Now Ridker and his colleagues have reported that statins work by lowering CRP levels and inflammation as well.

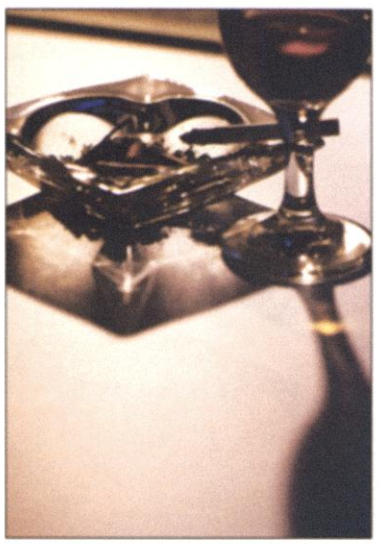
Last June, Ridker and his colleagues reported in *The New England Journal of Medicine* that statins reduced heart disease risk even in patients with otherwise healthy cholesterol levels, provided their CRP levels were above normal. "What was extraordinary," says Ridker, "was that the drug was just as effective in saving lives in the absence of high cholesterol, if the CRP [level] was high. Not only are these drugs 'anti-inflammatory,' as well as lipid lowering, but now there's actually clinical evidence to show that perhaps the way we prescribe these drugs needs to be rethought, because people with low cholesterol can still benefit from these drugs if they have an inflammatory response."

CRP as a Risk Factor

↑	total mortality
↑	heart attack
↑	stroke
↑	sudden cardiac death
↑	type II diabetes
↑	metabolic syndrome
↑	body mass index
↑	weight
↓	weight loss
↑	cigarette smoking
↑	heavy drinking
↑	no alcohol consumption
↓	drinking in moderation
↑	hormone replacement therapy in postmenopausal women
↑	age
↔	cancer
↔	cholesterol

To complicate the picture, CRP concentrations are associated not just with heart disease but also with a host of variables and risk factors for the disease. For instance, studies have shown that baseline CRP levels are very strongly linked to body mass index. "People lose 10 pounds," Ridker says, "and their CRP levels go down." CRP levels are also higher in patients who have type II diabetes or glucose intolerance, a condition related to diabetes. Higher levels of CRP are also associated with syndrome X, also known as metabolic syndrome, which increases the risk of diabetes, heart attack, and stroke and is characterized by excessive abdominal fat; insulin resistance; elevated blood pressure, blood sugar, and triglycerides; and low levels of the beneficial high-density lipoprotein cholesterol. Smokers also have significantly higher CRP levels, as do alcohol drinkers, although as Koenig and his colleagues have reported, that latter association is U-shaped: Heavy drinkers and nondrinkers have higher CRP levels than do those who drink one or two glasses a day. This is interesting, says Koenig, because the association between heart disease rates and alcohol consumption has proven to be U-shaped as well. Hormone replacement therapy seems to double CRP levels in postmenopausal women, which may help explain why hormone replacement therapy seems not to help stave off heart disease, as originally thought. To top it off, baseline CRP has been shown to increase with age.

This deluge of epidemiologic associations and clinical possibilities has left researchers struggling to make sense of it all—in particular, says Maseri, "to understand precisely through which mechanism CRP is contributing to cause myocardial infarction." Or as Pepys puts it, "there's no doubt now that CRP, as a marker of inflammation, is clearly related to arteriosclerotic, thrombotic events in an associative way. But it doesn't tell you anything about causality. Those are the facts; everything after that becomes speculation."



Risky business. On average, CRP levels rise with (orange arrows), fall with (green arrows), or are independent of (blue arrows) the incidence of other factors in a population.

Despite the swarm of research surrounding CRP and inflammation, scientists are still debating whether inflammation is a primary event that causes atherosclerosis or a secondary event, perhaps caused by the damage that is initiated by cholesterol deposition on the artery walls or even by smoking or high blood pressure. "CRP is caused by [heart] disease," says Ernst Schaefer, for instance, who studies lipid metabolism at Tufts University and the U.S. Department of Agriculture's Human Nutrition Research Center on Aging in Boston. "It's not the other way around."

But researchers studying CRP and inflammation directly point out that CRP levels do not seem to correlate with the actual extent of arteriosclerotic plaques, which all individuals begin to develop in their late teens. They believe that CRP levels reflect the body's response to inflammation elsewhere in the body, which in turn causes or exacerbates heart disease. Maseri compares CRP's role to that of fever, another response to infection. "Fever is beneficial because it stimulates the body to respond" by creating an environment inhospitable to the cause of the infection, he says. "But if your temperature goes too high—to 103° or 104° [Fahrenheit; 39.4° or 40°C] then you may die. So the response has to be appropriate to the infection: Too much is bad."

Case studies of individuals who are predisposed to malignant coronary arteriosclerosis, who lack particular risk factors but whose CRP levels are "off the map," suggest one possible mechanism by which CRP levels affect disease, says Ron Krauss, head of the department of molecular medicine at Lawrence Berkeley National Laboratory in California. Copious coincidental infections, such as chronic bronchitis, chronic prostatitis, or gingivitis, could lead to a low-grade, smoldering inflammatory response that could accelerate or initiate the development of arteriosclerosis and heart disease. The release of cytokines from these other infections prompts cytokine release in the artery wall, making pathological a relatively benign situation. This "echo phenomenon," as Harvard's Libby calls it, could be exacerbated in those individuals with a high genetic predisposition to inflammatory response. One theory that has not been supported in clinical studies is that the inflammation arises from direct infections by such bacteria as *Chlamydia pneumoniae* or *Helicobacter pylori*.

Many researchers seeking to make sense of these contradictions evoke evolution to explain the role of inflammation in heart disease, although the explanation, like virtually all in evolutionary biology, is difficult if not impossible to test. In prehistory, so their argument goes, most people died from trauma and infections; only the lucky few lived to see 30. As a result, evolution selected individuals with a genetic predisposition for mounting a heightened immune response. In the 20th century, as people started living well past their 50s, arteriosclerosis arose as a byproduct of that heightened immune response. In other words, says Vermont's Tracy, in an evolutionary sense, "we trade short-

CREDIT: (RIGHT) ROSS ANANIA/PHOTODISC

term benefit for long-term damage. And that's a trade that we're willing to make genetically, because we were never designed to live the long haul."

If the inflammation does indeed lead to heart disease and not the other way around, that still leaves open the question of whether CRP is simply a marker of inflammation or has its own pathological actions. In other words, is it an innocent bystander or a perpetrator of disease?

Over the past few years, biologists have accumulated considerable data suggesting that CRP is indeed a major player in the disease process. They have shown that CRP is present in arteriosclerotic lesions and that it functions as a chemoattractant to lure monocytes to the site. It has also been implicated directly in increasing the expression of adhesion molecules. It also apparently can activate immune system components known as "complement" proteins, which are important mediators of inflammation. What's more, as Pepys and his collaborators demonstrated in the early 1980s, CRP binds specifically to LDL cholesterol, the foamy stuff of arteriosclerotic plaques. There's also strong evidence that CRP can increase the uptake of LDL by macrophages to form foam cells and that CRP can enhance blood clotting, although that is still controversial. Finally, Pepys and his collaborators have shown that if you put human CRP into rats and then induce a heart attack, the attack is considerably more damaging than an attack induced without CRP, and the amount of heart muscle killed in the attack is greater by 40%. "CRP is clearly enhancing the size of the infarction in the rat model," says Pepys.

CRP is beginning to find its way into clinical medicine. Physicians have started to measure it in patients to assess heart disease risk: President Bush reportedly had his CRP level measured, for instance, and he was told it was fine, says Ridker. Research labs in academia, biotechnology, and the pharmaceutical industry are looking into the possibility of using molecules that inhibit CRP binding to reduce the risk of stroke and heart attack or perhaps to reduce the degree of damage afterward. They are also considering attacking other inflammatory mediators. "We're still left with the challenge of trying to sort out what's really important," says Libby.

The clinical payoff could be twofold. On the one hand, if the latest data stand up, says Libby, it means that plenty of asymptomatic individuals with no classical risk factors and low cholesterol levels but high CRP levels—perhaps one in every five Americans—are at high risk of heart attacks and could benefit from treatment, including statin drugs. "Our challenge is to learn how to treat these walking well who can benefit from statin therapy," says Libby. "We might want to use CRP or other markers of inflammation as a way of targeting therapy to these individuals as primary prevention." Indeed, in March the American Heart Association, the American College of Cardiology, and the Centers for Disease Control and Prevention co-hosted a meeting in Atlanta to develop clinical guidelines for when and how to use CRP measurements in treating patients.

The ultimate payoff is likely to come from identifying the ideal targets for inhibiting inflammation. That step, in turn, could eliminate at least some of the damage caused by arteriosclerosis. "We have our work cut out for us for the next dozen years," says Libby.

–GARY TAUBES

CREDIT: (RIGHT) E. SALA

MARINE ECOLOGY

Picturing the Perfect Preserve

Computerized tools help marine researchers map reserve networks that can pass ecological and political tests

NEW YORK CITY—Designing modern marine reserves demands a deft touch. Planners must balance the need to protect fragile marine environments against strong economic and political pressures to mine oceanic riches. It sounds like a job for an experienced diplomat, but ironically, a key tool for dealing with such challenges may instead be a computer.

A growing number of scientists are turning to new mapping software to help them design networks of marine reserves that are both politically viable and ecologically effective. The programs enable planners to test thousands of possible arrangements for achieving conservation goals, such as preserving fragile coral reefs or shielding vulnerable spawning fish from nets. Just as important, cybermapping may allow reserve advocates to sidestep potentially disastrous political conflicts by flagging areas where a protected zone might draw opposition from anglers or other economic interests.

Coral jewel. Researchers hope that new cybermaps will help preserve coral reefs and other habitats in the Gulf of California.

Such simulations recently allowed a U.S.-Mexican research team to pinpoint potential trouble spots for a proposed network of reserves in Mexico's Gulf of California. Australian researchers are applying the approach to patches of the Great Barrier Reef. Another group of scholars hopes to build models that will improve the effectiveness of one of the world's first major reserve networks, in the Bahamas.

"Marine reserve modeling is showing some big improvements over where we were just a few years ago," says Sandy Andelman of the National Center for Ecological Synthesis and Analysis (NCEAS) in Santa Barbara, California, who helped develop the tools. Their growing popularity, she says, reflects the fact that "there are more possible ways of conserving marine biodiversity than we can picture in our heads."

Speaking here last month,* marine ecologist Enric Sala of the Scripps Institution of Oceanography in La Jolla, California, described how the new tools can allow a small scientific team to draft a reserve plan for a large area quickly and relatively cheaply. Sala's target was the shallow water habitats of

* "Sustaining Seascapes: The Science and Policy of Marine Resource Management," American Museum of Natural History, New York City, 7–8 March.