HEART DISEASE

Army Targets a Potential Vaccine Against Cholesterol

Imagine going to your physician once a year for a shot that keeps your total blood cholesterol at safe levels—and thus helps ward off the accumulation of cholesterol in the dangerous artery-clogging plaques of atherosclerosis. With these simple injections, your risk of a heart attack or stroke, events responsible for more than half of all U.S. deaths, is slashed. To most cardiologists, the idea would sound like wishful thinking. But immunologist Carl Alving of Walter Reed Army Institute of Research in Washington, D.C, believes the scenario is not totally fanciful.

Alving and his co-workers have preliminary data from animal tests and other experiments suggesting that it may be possible to boost production of naturally occurring cholesterol-specific antibodies that they assert everyone possesses. If they are correct, anti-cholesterol "immunizations" of patients may one day supplant, or at least supplement, the difficult-to-follow diets and expensive drugs currently used to lower blood cholesterol.

BASIL RIFKIND, NHLBI

Blocked up. Some believe a vaccine may prevent clogged arteries like this one.

This unusual idea has excited some investigators. "If Alving's work is confirmed, it will represent an outstanding discovery. There's no question that high cholesterol levels lead to atherosclerosis, and that lowering cholesterol can reduce or limit it....For people who cannot do it with drugs or diet, antibodies could offer another option," says atherosclerosis researcher Robert Wissler of the University of Chicago. A Maryland company, EntreMed, is already working with Alving and his colleagues to exploit the research. "This offers a different way of thinking about a vaccine, that it can be used to fight a chronic disease that comes from within," explains John Holoday, the company's president.

Most of the cholesterol experts and atherosclerosis researchers contacted by *Science*, however, were either unfamiliar with the work—so far Alving's group has published only a few papers on the topic—or were intensely skeptical, or both. After having Alving's goals and results explained to him, for instance, atherosclerosis investigator David Via of Baylor College of Medicine said: "The concept doesn't make sense to me. There's a lot of questions that have to be answered before people will subscribe to this theory." Others, who didn't want to be quoted on the record, used words like "bizarre" and "far-fetched," questioning everything from the existence of anti-cholesterol antibodies to whether boosting production of such antibodies might actually prove more dangerous than beneficial.

Alving, whose more mainstream work on drug delivery and on vaccines for AIDS and malaria has garnered him a reputation as a careful and competent investigator, isn't surprised by the skepticism. Indeed, he's the first to admit that the notion of an anti-cholesterol vaccine is far from proven. He points out, however, that research supporting the approach actually has a long, if virtually unknown, history. He traces the first hints that anti-cholesterol antibodies exist to work done in Germany in the 1920s and 1930s. In the late 1970s, Alving says, his group essentially rediscovered the earlier German results in the course of its research aimed at using liposomes—tiny spherical particles composed primarily of phospholipids and cholesterol—as carriers for drugs and vaccines.

The Walter Reed lab found that liposomes containing high cholesterol concentrations —the most effective liposomes contained 71% cholesterol—activate the human complement system, a complex of proteins that helps the immune system respond to foreign antigens like invading microorganisms. Since the complement system is usually activated by antibody-antigen complexes, their data implied that the cholesterol-laden liposomes had stimulated antibody production.

Alving's lab followed up that observation, which was reported in 1977, with experiments in which they immunized mice with similar liposomes made only of phospholipids, cholesterol, and an adjuvant, a compound that helps trigger the immune system. The result: The mice generated antibodies that reacted with cholesterol. And in a 1988 paper in the *Proceedings of the National Academy of Sciences*, Alving and his colleagues reported success in using such mice to create monoclonal antibodies to cholesterol. The paper caused a stir at the time, but the results appear to have been widely disregarded since no one except Alving's lab seriously pursued the lead. Their next step was to determine if such anti-cholesterol antibodies occur naturally in humans. The answer, to their surprise, was a resounding yes. In a study of more than 800 people, says Alving, "essentially all the individuals" had such antibodies.

At first, this finding concerned the Walter Reed team, since such cholesterol-specific antibodies might scuttle plans to use liposomes as drug or vaccine carriers. Liposomes might, for example, trigger a harmful immune reaction or generate antibodies that destroy the liposomes. To determine the safety of the liposomes, the Army lab conducted a small study in which they injected the cholesterol-laden liposomes into rabbits that were then fed a cholesterol-rich diet. To their relief, the rabbits appeared unharmed by the immunization. What's more, rabbits treated with the liposomes showed a decreased tendency to develop high cholesterol levels. Ordinarily, high cholesterol diets send blood cholesterol concentrations soaring in rabbits, ultimately leading to the rapid formation of atherosclerotic plaques.

On the basis of these results, the Army agreed to fund a larger, better designed animal study. This most recent analysis has yet to be peer-reviewed and published, but Alving discussed the results last month in a talk at Boston's Center for Blood Research. He reported that in control rabbits fed a 0.5% cholesterol diet, serum cholesterol levels shot up from 50 milligrams per deciliter to 1500 and the animals had the expected high levels of aortic atherosclerotic plaques. But in rabbits immunized with these liposomes, cholesterol levels were 20% to 30% lower, and the number of plaques decreased by 30% overall, and by 80% in certain regions of the aorta. "I was surprised at the differences between the two groups," admits Fred Cornhill, whose team at the Cleveland Clinic did the computer image analysis of the plaques in the rabbit aortas and who was initially skeptical about the approach.

These early results have also caught the eye of Howard Kruth, chief of the National Heart, Lung, and Blood Institute's section on experimental atherosclerosis, who is now starting a collaboration with Alving and his colleagues. "It will be important to establish the generalizability of this observation in other model systems. It's intriguing, but there's a lot to sort out," he cautions.

Indeed, if Alving's work is to be taken seriously by most in the atherosclerosis community, he must satisfactorily answer a number of questions. A crucial one is whether the antibodies generated by the liposomes truly target cholesterol—and if they do, why don't they attack membranes throughout the body, since cholesterol is a major membrane

constituent. Or, as Alving poses the question: If we have antibodies to cholesterol, "why don't we disintegrate?" One possible answer, he suggests, is that cholesterol is rarely free in the body and is thus "masked" by other associated molecules; it may have to aggregate in unnaturally high concentrations before it can be recognized by antibodies. That, he says, might explain the observation that liposomes with less than 50% cholesterol rarely generate antibodies in mice.

Also unclear is the mechanism by which immunization with these liposomes clears excess dietary cholesterol from the blood and reduces plaque formation. Alving hypothesizes that the liposomes function in two ways. The antibodies they induce may tag the low-density lipoproteins (LDLs), the so-called bad cholesterol carrier, for clearance by scavenging macrophages. And the liposomes themselves may mimic the appearance of the LDLs, prompting cells to increase their number of LDL receptors and thus remove them from serum. In contrast, Kruth thinks the antibodies may have a different target: cholesterol-rich liposome-like particles he's found in atherosclerotic plaques.

At this stage, however, speculation in all areas far outstrips available data. In addition to follow-up animal studies, one future goal for Alving's team and EntreMed is the development of a simple, inexpensive test to gauge the levels of an individual's anti-cholesterol antibodies. Such an assay would be needed to help establish in a large population how levels of these antibodies correspond to total cholesterol levels, atherosclerosis progression, and overall health. If Alving's speculations prove correct, an individual's anti-cholesterol antibody level may be as important as blood pressure in determining the risk of heart attack or stroke. Alving himself finds comfort in the fact that he has a high level of these antibodies. "I certainly hope that it will be beneficial to me in the long run and protect me against heart disease. Data from the trials is giving me a lot of peace of mind," he told *Science*. It will be some time, however, before anyone knows if that solace is warranted or premature.

–John Travis

Boston-based freelancer P.J. Skerrett contributed reporting to this article.

PALEONTOLOGY

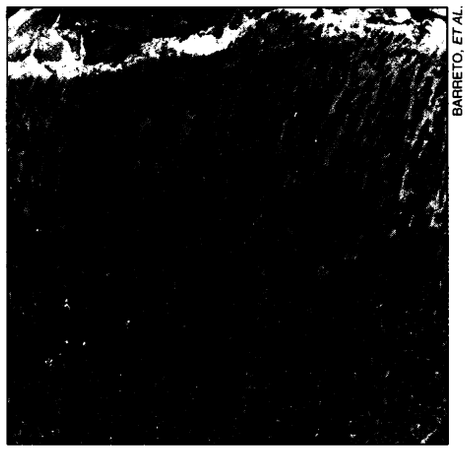
A Closer Look at the Dinosaur-Bird Link

Arrangements for family reunions, even for partly extinct families, rarely go completely smoothly. Over the past several years, dinosaur paleontologists have built a case that birds—not reptiles—are dinosaurs' closest living relatives by comparing the shapes of arms, shoulders, and claws. While this evidence is strong for one of the two main dinosaur groups, researchers have had difficulty fitting the second group into this family picture. Now comes evidence bringing that group into the avian fold, but the link is microscopic: the shapes of ancient cells.

Those cells belonged to a juvenile *Maiasaurus*, a duck-billed bipedal dinosaur—and a member of the disenfranchised group, known as the Ornithischia—that lived in Montana some 72 million years ago. On p. 2020 of this issue, paleontologist Claudia Barreto of the University of Wisconsin's School of Veterinary Medicine in Madison and her colleagues report that cells within the dinosaur's growth plates—discs of cartilage near the ends of bones that allow bones to grow—bear a striking resemblance to the cells of chicken growth plates. And they look very different from those of the growth plates of contemporary reptiles. "This work is very careful, very cautious, and very convincing," says paleontologist Kevin Padian of the University of California, Berkeley. "It means people can no longer say that dinosaurs are like reptiles because here they're doing things that we know only birds do."

Growth plates are made up of cells called chondrocytes. At the plates' boundary with the actual bone, chondrocytes die off, leaving behind their calcified extracellular matrix to serve as scaffolding for the osteoblasts (bone-forming cells) and blood vessels as they push into new territory. Using a light microscope, Barreto's team compared the *Maiasaurus* growth plates to those from a dog, a monitor lizard, and a chicken. They found that the plate-bone boundary of the dinosaur was very irregular, undulating up and down just as it does in contemporary birds. In contrast, the boundary zone in mammals and reptiles forms a straight line.

The team next found that the remnants of dinosaur chondrocytes themselves resemble those of birds. In mammals and reptiles, the cells are tall and have four distinct sides. In birds, the cells are shorter and ovoid in shape. That's the shape Barreto's group saw in the dinosaur plates. The researchers then used a scanning electron microscope (SEM) to peer into the extracellular matrix. In the *Maiasaurus* "the SEM showed calcified walls all around," Barreto says, as well as calcified lumps known as calcospherites. Again, this is identical to the pattern in birds and very different from what's seen in mammals and reptiles, who only have calcification and calcospherites on the longitudinal walls. "These growth plates point to a common ancestor for birds and dinosaurs. It's too complex to have evolved twice," Barreto concludes.



BARRETO, ET AL.

Growing likeness. Cells in this growth plate from a juvenile dinosaur bone resemble those from plates in birds.

Paleontologists who are more partial to reptilian relativity for dinosaurs, such as Larry Martin of the University of Kansas, argue that such a statement is too broad, and all Barreto has shown is a link between birds and the ornithischian branch of the dinosaurs. To include the other branch, the Saurischia, she will have to find this avian pattern in them as well. Barreto hasn't examined a juvenile saurischian yet ("That's the next step," she says), but she argues that many other features tie birds to saurischians. This, together with the growth plate evidence in ornithiscians, suggests to her that all the dinosaurs are related to birds.

And that general pattern not only links the ancient animals to modern avians, Barreto says, it also indicates the two dinosaur branches had one common ancestor. It had been argued that the two branches emerged separately from a diverse group of primitive reptiles called thecodonts. But here even paleontologists who favor the common ancestor theory think Barreto hasn't done the right comparisons to support that claim. "You need to look at crocodiles," says Jacques Gauthier of the California Academy of Sciences.

Crocs, next to birds, are presumed to be dinosaurs' nearest living relatives, deriving from that same general pool of ancient reptiles. If the birdlike growth plates are missing in crocs, Gauthier says, it implies the pattern arose in a dinosaur ancestor after the croc lineage went its own way. But if crocs do have these plates, the feature must have been older and more generalized, and says nothing about a common ancestor for the two dinosaur groups. So Barreto is off on another big game hunt, this time for crocodiles, but once again she's looking for something very small.

–Joshua Fischman