

IMMUNOLOGY

## The New View of Complement

This cascade of immune proteins has more diverse roles, and can cause more problems, in the body than once thought

If you visited immunologist William Robinson's lab at the Stanford University School of Medicine in Palo Alto, California, a couple of years ago, you might have found his postdoc Qian Wang operating on the knees of mice, performing the same surgery that many athletes undergo to repair a torn meniscus. No, the animals hadn't hurt themselves by running too vigorously on their wheels. Instead, the researchers were testing an unorthodox hypothesis about the cause of osteoarthritis (OA), the painful and sometimes crippling joint degeneration that strikes many of us as we age.

The standard explanation for OA attributes it to the gradual erosion of our joints over decades, but there have long been hints that something else is involved. The autoimmune disease rheumatoid arthritis, another condition that impairs the body's joints, stems from inflammation triggered by the immune system, and the joints of OA patients often show milder inflammation. Researchers haven't been sure, however, whether inflammation drives the damage of OA or is a byproduct of it.

To find out, Robinson's team began operating on multiple strains of genetically engineered mice that lacked various

inflammation-promoting genes, carving away some of their knee cartilage because that typically induces OA. (Athletes who have meniscus surgery frequently develop the arthritis.) The researchers got a jolt when they performed the surgery on mice lacking genes for the complement system, a cadre of immune proteins that researchers didn't think was a factor in OA. Rodents lacking either of two complement proteins incurred about 50% less knee damage than did control animals. And as the team reported last December in *Nature Medicine*, mice missing a complement-inhibiting protein showed more severe erosion. A role for complement in OA is an intellectual leap. "Everyone thinks that OA is simple wear and tear in the joint," Robinson says. "Complement may play a crucial role in the breakdown of cartilage and destruction of the joint in OA."

Arthritis researchers aren't the only scientists recently taken aback by new insights into the complement system. Again and again, it has confounded expectations, proving to be more versatile and powerful than anyone thought. Not that long ago, most researchers agreed that, as its name suggests, complement was a mere helper for immune cells. But then further research demonstrated

that the system is one of our most important protections against pathogens, killing invaders before other immune defenses have a chance to mobilize.

Even more unexpected, some researchers say, is the increasing evidence that complement components perform functions outside the immune system. Complement takes part in the body's growth and maintenance, for example. Recent work suggests it guides development of the brain and skeleton and spurs damaged organs to repair themselves. "The term 'complement' is a misnomer," says immunologist John Lambris of the University of Pennsylvania's Perelman School of Medicine.

But if complement does a lot of good in the body, it can also do us harm. "It's an essential component of normal physiology and pathophysiology," says Lambris, who notes that researchers have implicated the system in more than 30 illnesses. The list includes diseases and conditions known to have an immune connection—such as sepsis, rheumatoid arthritis, and organ transplant rejection—and ones that scientists didn't consider immune system diseases, such as OA and age-related macular degeneration (AMD), the leading cause of blindness for older people in

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**Stealing vision.** Drusen, the small, milky blotches on the retina of a patient with age-related macular degeneration, carry proteins from the complement system.

the developed world. The recent work “redefines these degenerative diseases as having a significant immune component and opens up new avenues for treatment,” Robinson says.

Already, two drugs have been approved for complement-related diseases, and several other compounds targeting proteins in the cascade are in clinical trials for a variety of conditions. “That’s pretty remarkable, and it’s just the beginning,” says immunologist John Atkinson of Washington University School of Medicine in St. Louis, who has studied complement for more than 40 years.

### Standing guard

Complement belongs to the innate arm of the immune system. Unlike the adaptive immune system that includes B and T cells, the innate arm doesn’t for the most part customize its defenses for specific pathogens. Complement was one of the first immune defenses recognized; at the end of the 19th century, researchers discovered that blood serum contained a bacterium-killing component in addition to antibodies. Complement was also one of the earliest defenses to evolve: Only vertebrates can muster B cells and T cells, but even sponges boast complement proteins, Lambris notes.

Complement usually leads the body’s counterattack against bacteria. In contrast to the adaptive immune system, which can take days or even weeks to reach peak performance, complement is always ready for action, and it dispatches invaders swiftly. “It’s an amazing first responder,” Atkinson says. “It can lyse a bug in 30 seconds.”

A sign of complement’s importance to our survival is that it accounts for about 4% of the proteins in our blood. Among the more than 30 types of complement proteins are danger detectors, activators that switch on other proteins, and inhibitors that curb self-directed attacks. They fall into three interconnected pathways (see figure). Those proteins on the lookout for potential threats are constantly checking the blood and scanning the surfaces of our cells. The complement system has several options once it detects a pathogen or other danger. In the most dramatic response, the complement component C5b and other

proteins convene to form a membrane attack complex, which lands on the surface of a microbe and pierces its membrane.

Stimulating complement can also spur defensive cells such as macrophages to eat an intruder and crank up inflammation, another protective measure. Complement is so good at its job, says immunochemist Robert Sim of the University of Oxford in the United Kingdom, that you usually aren’t aware it’s working; it kills off invading bacteria before they have the opportunity to make you sick.

Another of complement’s crucial roles involves searching out dying body cells and molecular junk. Complement proteins tag but don’t remove the refuse—they hail a macrophage or other cell to clean up—and autoimmune diseases such as lupus may result from the failure of complement to help eliminate this debris.

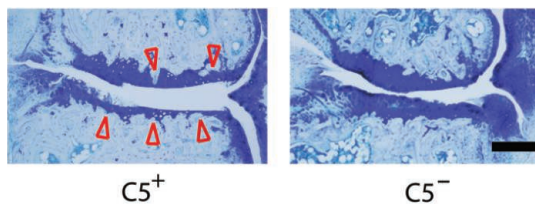
### Growth and regrowth

Keeping the body safe is complement’s traditional job, so tidying up potentially dangerous cellular flotsam isn’t out of charac-



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STANFORD UNIVERSITY  
SCHOOL OF MEDICINE



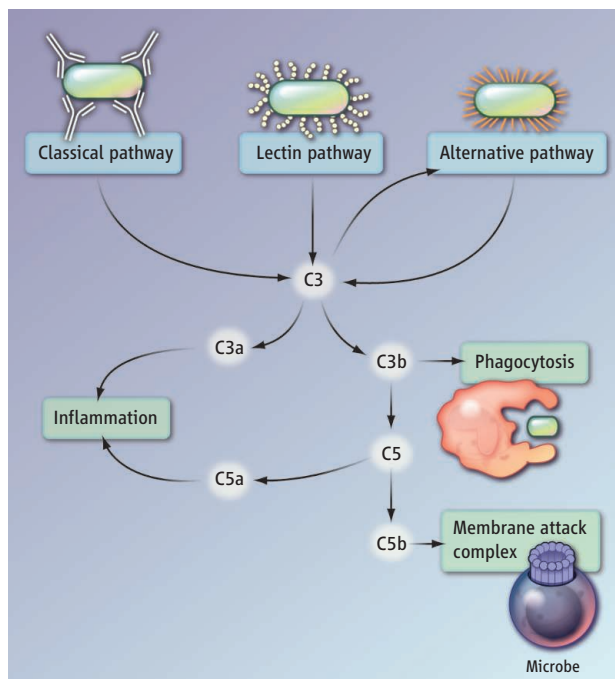
**Breaking down.** The knee of a control mouse shows more arthritic erosion (*left*, arrows) than one of a mouse that lacks the complement protein C5 (*right*).

ter. But recent revelations that complement helps steer normal development and fosters the repair and regeneration of damaged tissues have stretched our view of its contributions. Complement’s role in development is “the most striking” of its newly uncovered abilities, Sim says.

Several years ago, for example, a study led by neuroscientists Ben Barres of Stanford and Beth Stevens, now at Harvard Medical School in Boston, suggested that complement helps cut away unnecessary synapses during brain formation in young mice (*Science*, 14 December 2007, p. 1710). Earlier this year in *Neuron*, Stevens, Barres, and colleagues revealed how, showing that the complement protein C3 helps spur microglia, immune cells in the brain, to eat the unwanted connections.

Other studies point to developmental roles outside the nervous system. Last year, a multinational team of researchers reported that mutations in two complement genes were behind 3MC syndrome. Because children with this rare condition have facial deformities as well as learning disabilities, the finding indicates that complement helps shape the skeleton.

Another aspect of complement’s softer side is its role in the restoration of damaged tissues and organs. Unlike most other organs, the liver can regenerate after an injury. The



**Lines of attack.** This simplified diagram traces the main pathways of the complement cascade.

## Stalling Sepsis?

Unlike osteoarthritis and age-related macular degeneration, sepsis is an illness in which you'd expect complement to be involved. Typically triggered by a bacterial infection that sends the immune system into overdrive, sepsis involves runaway, body-wide inflammation, with complement at the heart of the process. "We've found that C5a [a complement protein] plays a major role in sepsis," says immunopathologist Peter Ward of the University of Michigan Medical School in Ann Arbor.

More than 10 years ago, Ward and colleagues showed that dosing rodents with an antibody that sticks to C5a spares them from sepsis. In subsequent experiments in which they blocked the C5a receptors in mice and studied animals that lacked these molecules, Ward's team discovered how C5a makes trouble. In 2008 the researchers reported that C5a triggers a surge in the immune system signals known as cytokines, unleashing the so-called cytokine storm that can spur numerous organs in the body to stop working.

Several companies have begun testing C5a inhibitors for diseases such as atherosclerosis, and targeting the same molecule could be therapeutic for sepsis patients. Researchers are desperate for good news about the condition. More than 40 clinical trials of sepsis treatments have already failed, Ward notes, and the only drug approved in the United States specifically for sepsis, activated protein C, has been withdrawn from the market because of new evidence it doesn't work. Doctors can only offer general measures—such as broad-spectrum antibiotics and artificial respiration—that don't provide much benefit. In the United States, sepsis is fatal for almost 30% of the 750,000 people who fall victim to it each year. "It's a very frustrating situation right now," Ward says.

—M.L.

liver also manufactures most of the body's complement proteins—and the two capabilities seem to be related. "We have found that complement-deficient mice have impaired liver regeneration," Lambris says. An injury, such as one caused by a liver-damaging chemical, spurs production of the complement proteins C3a and C5a. The molecular details of how these proteins prompt the liver to refurbish itself remain unclear, Lambris says. But 3 years ago, he and his colleagues discovered that the proteins help keep dividing liver cells alive by activating a protective pathway.

### The price of vigilance

Then there's the dark side of the complement system. "You need it, and if you don't have it you either get infections or you develop autoimmunity," Atkinson says. "But you don't want to turn it on a healthy cell."

Researchers are uncovering more and more instances in which that occurs. For example, complement attacks might take years off the working lives of transplanted organs. The cascade triggers much of the damage from so-called ischemia-reperfusion injuries, which occur after blood flow to a tissue or organ is temporarily cut off—such as by a blood clot or removal of the organ from a donor's body in preparation for transplantation. The two complement proteins C5a and C5b are the main culprits. C5a fires up damaging inflammation by stimulating immune cells known as neutrophils. Meanwhile, C5b and other proteins form membrane attack complexes that kill cells in the donor organ.

The effects of complement typically aren't severe enough to prevent a newly transplanted organ from working, says transplant immunologist Steven Sacks of the MRC Centre for Transplantation at King's College London. But all transplants eventually fail, and complement could hasten that process. "The question is why a 40-year-old organ doesn't last another 30 years," Sacks says.

He and his colleagues have developed a possible way to reduce complement-induced damage. The premise is that "the fate [of a transplanted organ] could be sealed based on the amount of reperfusion injury," Sacks says. So before implanting the organ, the research-



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ers wash it with an engineered artificial protein called mirococept, which sticks to cells in the organ and blocks all three branches of the complement system. The team has already completed a safety study of the compound in people, as well as a second study in 12 kidney transplant patients that produced encouraging preliminary data that the wash protected the donor organs from ischemia-reperfusion injury. Sacks says that a larger trial of mirococept will begin later this year at about 14 kidney transplant centers in the United Kingdom.

### Hard on the eyes

Whether mirococept will prove itself in these trials remains to be seen, but some people with rare diseases are already benefiting from recent complement discoveries. The U.S. Food and Drug Administration has approved two anticomplement treatments. One is the antibody eculizumab, which latches onto the complement protein C5 and blocks the subsequent cascade. Doctors can now prescribe it for atypical hemolytic uremic syndrome, in which complement attacks the kidneys, and paroxysmal nocturnal hemoglobinuria, in which complement destroys blood cells. The second drug, Cinryze, blocks an enzyme in the complement cascade and ameliorates hereditary angioedema, in which out-of-control complement activity can cause symptoms such as swelling of the limbs and difficulty breathing.

Researchers predict that targeting complement will translate into other treatments. One disease that has already drawn a large amount of interest from scientists and drug companies is AMD. A combination of biochemical sleuthing and genome crunching connected complement to this macular degeneration, which usually strikes the eyes of people after age 50. In the disease, the portion of the retina that provides sharp vision deteriorates, often obliterating the central part of the visual field and leaving people unable to drive or read.

In the late 1990s, Gregory Hageman, now at the University of Utah School of Medicine in Salt Lake City; retinal cell biologist Don Anderson of the University of California, Santa Barbara; and colleagues decided to determine what was in the small globs of material called drusen that blemish the retinas of AMD patients. Thanks to Hageman, who

was then at the University of Iowa in Iowa City, the researchers had access to plenty of eyes that had been donated to the university to provide corneal transplants; once the corneas were removed, the eyes were usually thrown away.

At first, the results of the analysis were puzzling, Anderson recalls. The initial protein the scientists identified in drusen was vitronectin, which, among other roles, naturally inhibits the activity of complement's membrane attack complex. Anderson says the team kept the findings under wraps for 2 years: "We were sitting around scratching our heads." But further probing of drusen revealed other complement proteins.

The case for complement's involvement in AMD grew stronger when researchers began checking for gene variants that were more common in patients with the eye disease. In 2005, four groups, including Hageman and Anderson's, reported that variants in the gene for factor H, a key complement inhibitor, boosted the risk of developing AMD. Researchers have since discovered that alterations in just three complement-related genes, including the one for factor H, account for about 75% of AMD cases in the developed world.

Before this work began, scientists ascribed AMD's retinal damage to factors such as smoking and high levels of blood lipids and "had no suspicions it was an immune disease," notes ophthalmologist and eye researcher Robyn Guymer of the University of Melbourne in Australia, who wasn't involved in the studies. "It really changed everyone's thinking about where to look in AMD." In a review published earlier this year, Guymer tallied the results of that change in perspective: At least eight complement inhibitors, including eculizumab, are undergoing preclinical or clinical testing for AMD.

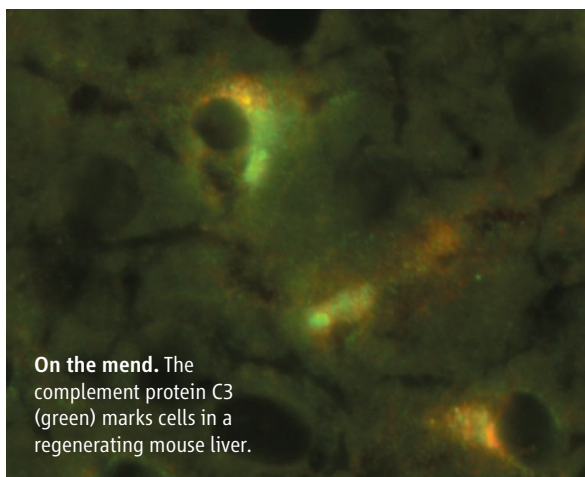
### A drug problem?

Having two approved drugs for complement-related conditions is encouraging, researchers say. But both drugs have drawbacks, particularly their cost. A year's worth of eculizumab runs more than \$400,000, and Cinryze isn't much cheaper.

One possible route to more economical alternatives, Lambris says, involves small peptides that would be easier to manufacture. He and his colleagues have synthesized a molecule called compstatin that suppresses C3, the hub of the complement cascade. "We feel this is a good way to prevent complement

activation," says Lambris, whose university licensed the compound to a biotech company for further development. One benefit of interdicting the cascade at C3, he notes, is that it prevents complement from continually churning out compounds that switch on inflammation-promoting neutrophils. Lambris adds that several lines of evidence, including studies of other C3 inhibitors, suggest that this strategy is safe. Phase II trials, run by a second pharmaceutical company, are evaluating a modified version of the compound for AMD.

With more than 30 proteins, the complement system seems to offer plenty of targets for drug designers. But compstatin highlights one of the tricky questions in complement drug design: how to tamper with the cascade without subverting its antibacterial abilities. For example, some researchers worry that blocking C3 will prevent production of the key defender C3b, which spurs macrophages and other phagocytic cells to devour invaders. "If you inhibit complement early, ... you



**On the mend.** The complement protein C3 (green) marks cells in a regenerating mouse liver.

will seriously compromise innate immune function," says immunopathologist Peter Ward of the University of Michigan Medical School in Ann Arbor.

To limit possible side effects, some researchers favor concentrating on proteins further down the complement cascade. Eculizumab, for instance, targets the C5 protein. But Ward says he's concerned that even blocking C5 would leave people vulnerable to microbes; he notes that patients are required to get vaccinations against meningitis bacteria before receiving the antibody. Activated C5 splits into C5a, which ignites inflamma-



**Changing places.** Removing an organ for transplantation unleashes complement-mediated damage.

tion, and C5b, which joins the membrane attack complex that slays bacteria. A better alternative, Ward says, is inhibiting C5a. His lab has been investigating whether a C5a-disabling antibody is beneficial for sepsis in animals (see sidebar).

Immunologist Michael Holers of the University of Colorado, Denver, and colleagues have taken a different approach to minimize the collateral damage of interfering with complement. They devised a combo molecule that includes part of a complement receptor—a protein that enables our cells to respond to complement proteins—and part of the complement inhibitor factor H. The idea is that the drug, dubbed TT30, will home in on tissues where complement is active. The receptor portion of TT30 sticks to any of our cells that are under complement attack and allows the inhibitor to shield them from the onslaught, but the compound isn't a general immunosuppressant because it doesn't inhibit complement throughout the body. Now being developed by a pharmaceutical company, the drug has made it through Phase I safety trials, Holers says.

We might even find ideas for new complement therapies within our worst enemies, Lambris says. Human pathogens have fought an evolutionary battle against the complement system for hundreds of millions of years, and they've come up with some devious tricks to evade it. For example, *Staphylococcus* bacteria produce at least eight complement inhibitors that could serve as templates for new drugs, he says. Knowing our enemies better might help protect us from the friendly fire of one of our strongest defenses. —MITCH LESLIE