

Challenges in Immunology

cells could at a minimum leave people with enough of an immune system to ward off serious disease.

At the nearby City of Hope in Duarte, California, John Rossi heads a study that's recently started enrolling patients in the most aggressive HIV gene therapy yet. In five people with AIDS lymphoma, a cancer of the lymph nodes, Rossi, John Zaia, and colleagues will use various chemotherapies or radiation to completely destroy each person's immune system—a dangerous procedure that is the standard of care for that highly lethal condition. The researchers will then infuse the patients with their own previously harvested immune stem cells that an HIV-based vector has transduced with three genes. The therapeutic genes encode a ribozyme that knocks down CCR5, a short RNA that interferes with the virus's ability to copy itself, and a decoy that codes for an essential HIV protein and throws a wrench in the viral replication machinery. "The nice thing is, the targets are multiple," says Rossi, who hopes this will overcome a risk in all these strategies—namely, that HIV will develop resistance to the gene therapy.

Instructive immunotherapy

At the California Institute of Technology in Pasadena, David Baltimore has teamed up

with immunologist Pamela Björkman on an HIV gene-therapy project that he calls "instructive immunotherapy." Rather than bolstering the natural immune response, Baltimore says, "we're instructing the immune system [about] what to make."

This 5-year experiment lives up to its Grand Challenges billing with its focus on inventing virus-fighting antibodies. Gene therapists have paid antibodies little heed because HIV notoriously remains impervious to their attack. "I didn't think we should be giving up on the historically most powerful part of the immune system," says Baltimore. So he and Björkman are attempting to construct an antibody against HIV that's far more powerful than anything naturally produced by the immune system. Baltimore and co-workers then want to use an HIV-based vector to transduce the gene for this antibody into immune stem cells.

Baltimore originally explored intracellular immunization strategies—he even coined the term—but his work now on instructive immunotherapy reflects a belief that multiple forms of gene therapies may be needed to defeat HIV. "I'm hedging my bets," says Baltimore.

Two years into the project, Baltimore says his team is making steady progress, but

they have an added hurdle to overcome. They need to craft antibody genes that will continue to function as the CD34⁺ stem cells mature into the B cells that ultimately secrete the antibody. Within 3 years, the scientists hope to show that this can work in chimeric mice that have humanlike immune systems. "We're very aware that this is complicated and expensive and difficult to imagine using in the less developed world," says Baltimore, noting that the Gates initiative demands that researchers work on projects applicable to the world's poor. With that in mind, Baltimore says they've been testing another strategy in mice: injecting the vector directly into the body to see if it will home in on CD34⁺ cells.

In the end, Baltimore and other researchers in the field imagine that different gene therapies and anti-HIV drugs will complement each other. And many anticipate that in wealthy countries, demand for a gene-therapy approach will grow as ever more people become resistant to the best anti-HIV drugs available. "With the right techniques and vectors, I think this can be just like what the Red Cross does with blood transfusions," predicts the University of Pennsylvania's June. "Unfortunately, it's going to take time."

—JON COHEN

NEWS

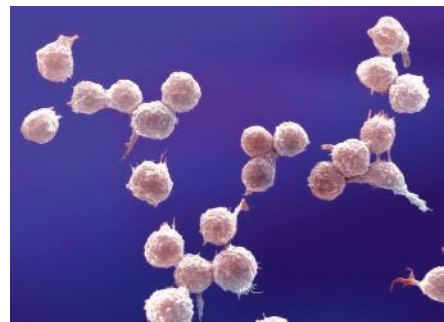
Mast Cells Show Their Might

They are the most reviled cells in the body. Their meddling makes our skin itch, our eyes swell, and our noses stream; the cells even provoke suffocating asthma attacks that kill thousands of people every year. In fact, these villains, known as mast cells, are responsible for so much suffering that some researchers have proposed eradicating them.

That could be a big mistake. Over the past decade or so, the reputation of these immune cells has been turned around. Researchers have learned that mast cells are vital sentinels that orchestrate counterattacks on invading bacteria and viruses. The cells link the innate immune system, which deploys a standard set of defenses, with the adaptive immune system, which customizes the body's weapons to a specific attacker. Mast cells even neu-

tralize toxins from snakebites and bee stings (*Science*, 28 July 2006, p. 427).

However, mast cells turn out to be fickle allies. Extending the cells' disease connections far beyond allergic reactions, recent studies put them at the center of multiple



Standing guard. Mast cells from the umbilical cord.

Once dismissed as "allergy cells," mast cells have proven crucial for immunity. But they've also shown a dark side

sclerosis, rheumatoid arthritis, cancer, and atherosclerosis. "What this research tells you is that mast cells are key to a lot of biological processes," says immunologist Dean Metcalfe of the National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland.

The catalyst for many of these discoveries was the identification of mutant mice that lack mast cells. A white-spotted coat on one of these rodents first attracted geneticists' attention in 1937. But it wasn't until the late 1970s that Yukihiko Kitamura of Osaka University Medical School in Japan and colleagues determined that the genetic defect responsible for the color change also short-circuited mast-cell development. Led by Kitamura and pathologist Stephen Galli of Stanford University in Palo Alto, California,

researchers began using the animals in the late 1980s to probe mast-cell activity. By implanting these mice with lab-grown mast cells, scientists could finally begin to elucidate the functions of the cells or parts of their molecular repertoire. The animals are “the only way to see if the effect [of mast cells] is positive, negative, or neutral,” says Galli.

The good

Mast cells are microscopic chemical factories. Their product line of 10,000 compounds includes histamines that make blood vessels leaky, protein-slicing enzymes such as chymase and tryptase, and cytokines that incite inflammation and activate immune cells.

These potent potions, which the cells often stash in sacs called granules, cause misery for people who are sensitive to cat dander, ragweed pollen, and other allergens. The trouble begins when antibodies jutting from mast cells latch onto one of these substances. Once triggered this way, the cells dump their contents, or degranulate. This outpouring can elicit responses that range from local irritation, such as stuffed noses and hives, to bodywide and potentially fatal anaphylaxis. Scientists once thought these woes were a side effect of mast cells protecting us from parasitic worms, and that allergic reactions ensued when the cells instead overreacted to innocuous substances.

That rationale for the cells’ existence satisfied few researchers because the costs seemed to dwarf the benefits. “Everyone knows that they make people itch, sneeze, and wheeze, but why are they there?” asks immunologist George Caughey of the University of California, San Francisco.

A pair of papers published in *Nature* in 1996 marked the turning point in the thinking about mast cells. One came from microbiologist Soman Abraham of Duke University in Durham, North Carolina, and co-workers and the other from Bernd Echtenacher of the University of Regensburg in Germany and colleagues. The teams were the first to test the bacteria-fighting prowess of mice lacking mast cells. Both groups unleashed internal infections in the animals by introducing bacteria into the peritoneal cavity. And both groups found that mice with mast cells could beat back the invasion, whereas rodents devoid of the cells died.

The findings shook up the field because they were the first to show that the cells were “life-or-death critical” for fighting infection, says Caughey.

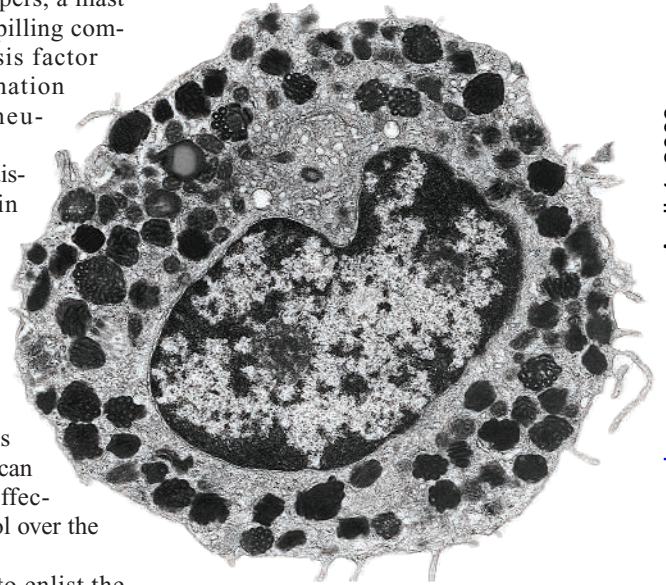
Further research has demonstrated that innate immunity depends on the mast cell taking on a range of bacteria and viruses. “It’s the quintessential immunological first responder,” says Joshua Boyce of Harvard Medical School in Boston. Mast cells reside just about everywhere a pathogen might try to break in: the skin, nasal passages, lungs, lining of the gut. Their membranes are studded with bacteria-sensing proteins called Toll-like receptors. Although it can gobble interlopers, a mast cell typically fights back by spilling compounds such as tumor necrosis factor (TNF) that promote inflammation and lure pathogen-killing neutrophils to the infection site.

Researchers have also been discovering surprising subtlety in mast-cell responses. “The misconception was that they are a bag of activators that goes pop,” says immunologist Jean Marshall of Dalhousie University in Halifax, Canada. But research by her lab and others revealed that activated mast cells don’t always degranulate. They can dole out cytokines and other effectors, giving them precise control over the behavior of other cells.

Mast cells use that power to enlist the adaptive immune system, whose soldiers are B and T lymphocytes. Before these cells join the battle, however, so-called helper T cells must rendezvous with a cell sporting a fragment, or antigen, of the invader. As Abraham and colleagues showed 4 years ago, mast cells promote encounters between antigen-presenting cells and helper T cells by inducing one of the most familiar symptoms of infection: swollen lymph nodes. Mast cells that have detected a pathogen emit TNF, which spurs nearby lymph nodes to bulk up and release molecules that draw in lymphocytes. And last year, Galli and colleagues reported that TNF from mast cells prods one antigen-presenting cell, known as a dendritic cell, to make a beeline for the nodes, where it can mingle with T cells. “In the absence of mast cells, these events don’t occur, and you get a poor response from the immune system,” says Abraham.

Randolph Noelle of Dartmouth Medical School in Lebanon, New Hampshire, and colleagues have also implicated the cells in immunological tolerance, in which the immune system learns not to assault the body’s own tissues. Researchers knew that

so-called regulatory T cells play a key role in the development of tolerance by quashing immune attacks, but they didn’t know how. Last August, in an article that appeared in *Nature*, Noelle’s team showed that mast cells serve as enforcers for regulatory T cells, turning down the immune system’s reaction to skin grafts. That an “innate” cell is so deeply involved in this adaptive response is surprising, Noelle says.



Loaded. Granules full of histamine and other effector molecules fill a mast cell.

The bad

The upside of mast cells is bigger than anyone imagined a decade ago. But so is their downside. Work by rheumatologist David Lee of Harvard Medical School, for example, indicates that mast cells help initiate rheumatoid arthritis. Five years ago, Lee and colleagues reported that mice lacking mast cells don’t develop the rodent equivalent of this debilitating condition (*Science*, 6 September 2002, p. 1689). In further studies, the researchers have found that activated mast cells pump out the cytokine interleukin-1, which attracts inflammation-inducing cells to the joint. Mast cells also promote leakage of fluid into the joint, which allows in more self-targeted antibodies that might lead to damage by a cadre of defensive proteins known as complement.

In multiple sclerosis, mast cells may worsen the disease’s destruction of the myelin insulation that sheathes nerves—at least if experiments on the rodent model of the condition, known as EAE, hold true for humans. Seven years ago, immunologist

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Melissa Brown of Northwestern University's Feinberg School of Medicine in Chicago, Illinois, and colleagues determined that although EAE mice still develop disease if they have no mast cells, the symptoms start later and are less severe. Brown says she assumed that mast cells in the central nervous system (CNS) exacerbated the illness. But in 2003, her group found that symptoms aren't delayed in mice lacking mast cells only in the CNS. The culprits, Brown now suspects, are mast cells hiding in the spleen and lymph nodes, where they could help activate self-destructive T cells that then travel to the CNS and target myelin.

Rogue mast cells might also contribute to the developed world's leading killer, heart disease. The cells infiltrate arterial gunk, although they are outnumbered there by macrophages and smooth muscle cells. More than 20 years of in vitro experiments by cell biologist Petri Kovanen of the Wihuri Research Institute in Helsinki, Finland, and colleagues link mast cells to plaque formation and breakage, the event that triggers most heart attacks. For example, mast cells might prompt flimsy capillaries to grow into the fatty buildup and then bleed, helping to weaken the plaque.

Biochemist Guo-Ping Shi of Harvard Medical School and colleagues have now taken this investigation into animals. In a *Nature Medicine* article published in June, they report the first data on artery clogging in mast cell-deficient mice. The animals had been crossbred with rodents that are atherosclerosis-prone. Plaques in mice without mast cells were not only smaller than ones in animals with the cells but also less likely to fracture, Shi's group found. The scientists pin the blame on proteins called cathepsins, which cause trouble in several ways. For instance, mast cells spur neighboring cells to release these enzymes, which dissolve proteins in the blood vessel walls, allowing additional smooth muscle cells to escape into the plaque.

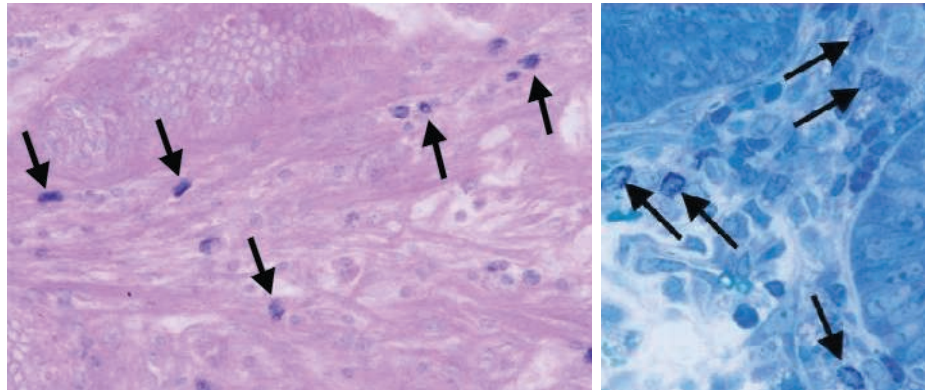
Mast cells can break the heart in another way. Last year, physiologist Randi Silver of Weill Medical College of Cornell University in New York City and colleagues temporarily cut off blood flow to rodent hearts, simulating a heart attack. If the mice had mast cells, their hearts were more likely to display abnormal rhythms—including the often-lethal ventricular tachycardia—after blood flow resumed.

The ugly

Researchers have been trying to pin down mast cells' role in cancer since the German

microbiologist Paul Ehrlich, who named the cells in the late 1800s, noticed that they swarmed around tumors. Sometimes mast cells appear to oppose the growths and sometimes to abet them.

There are more examples of the latter. Cancer biologist Lisa Coussens of the University of California, San Francisco, and colleagues



Ally or adversary? Mast cells (arrows) take up residence in colon tumors.

have found, for example, that skin tumors exploit mast cells' proclivity for cleaning up. After an injury, mast cells release compounds that help dissolve damaged tissue. They also promote repair by, among other things, spurring angiogenesis. These actions benefit cancer cells, piping in nutrients and speeding growth. Indeed, 8 years ago, Coussens and colleagues showed in mice that the absence of mast cells hinders the progression of precancerous skin growths into tumors.

Recent results from immunologist Gunnar Nilsson of the Karolinska Institute in Stockholm, Sweden, and colleagues suggest an even more insidious partnership in Hodgkin's lymphoma, a cancer in which abnormal immune cells multiply out of control. The tumor cells get a jolt when the CD30 receptor on their surface couples with a molecule on the surface of a mast cell. The interaction also stimulates the mast cell, which releases compounds that spur inflammation needed for tumor growth.

In general, mast cells have turned out to be more subtle than scientists expected. They can perform opposing functions—for example, igniting and quenching inflammation. And as Galli notes, mast cells sometimes have contrasting effects at different times in the same process.

Their upbringing seems to determine their behavior. Unlike blood cells, which circulate and tend to be uniform, mast cells specialize for their home tissue, says immunolo-

gist Juan Rivera of the National Institute of Arthritis and Musculoskeletal and Skin Diseases in Bethesda, Maryland. The result is a multitude of varieties, each of which "responds to and is tailored to the tissue it develops in," says Lee. Researchers are just beginning to probe how a mast cell's milieu shapes its function.

Most work on mast-cell function carries a key caveat: The subjects were rodents. Understanding of the human cells has lagged. One reason, says Boyce, is that "there's no such thing, that we know of, as a mast cell-deficient human." Still, researchers are considering therapies that target mast cells. And that could be tricky.

Turning down mast cells could leave patients vulnerable to infection. Turning them up could prompt fatal anaphylaxis. Nonetheless, researchers are exploring the possibility that allergy and asthma drugs directed at mast cells or their contents—even good old antihistamines—will work against other conditions. One example is cromolyn, a mast-cell stabilizer found in some asthma inhalers. Shi and colleagues are testing its power against atherosclerosis in mice. And last year, in the *Journal of the National Cancer Institute*, Craig Logsdon of the University of Texas M. D. Anderson Cancer Center in Houston and colleagues reported that the inhibitor curtails growth of mouse pancreatic tumors. The researchers are now planning a clinical trial.

Plenty of mysteries remain about the Jekyll-and-Hyde nature of mast cells. But on the question of whether we can live without them, Galli says he knows where he stands: "If somebody told me I could have all my mast cells eliminated tomorrow, I wouldn't agree to that."

—MITCH LESLIE