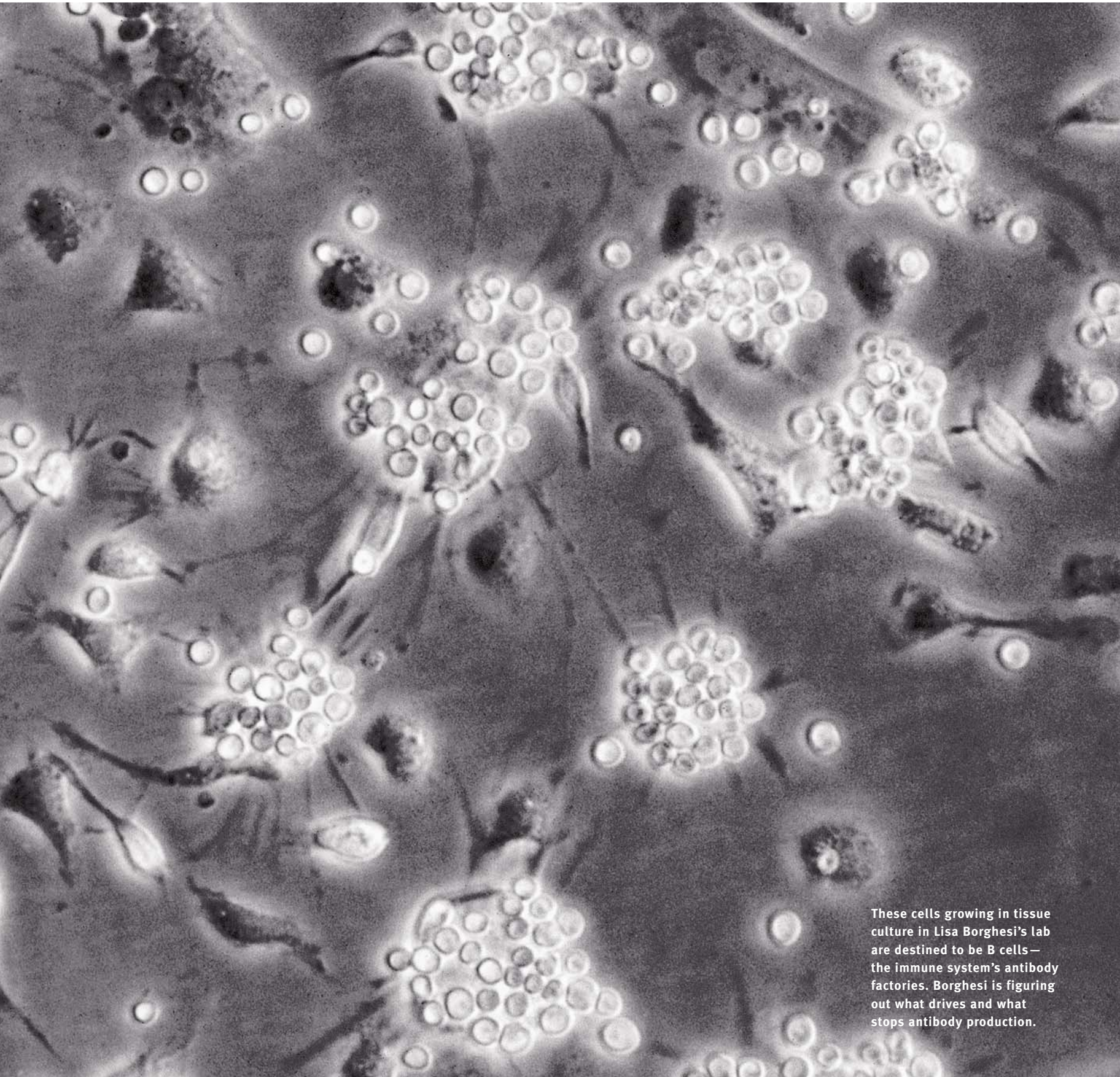


INVESTIGATIONS

Explorations and revelations taking place at the medical school



These cells growing in tissue culture in Lisa Borghesi's lab are destined to be B cells—the immune system's antibody factories. Borghesi is figuring out what drives and what stops antibody production.

BUBBLES ASIDE

HOW GENE THERAPY MIGHT BECOME A VIABLE OPTION
FOR A RARE IMMUNE DISEASE | BY KRISTIN OHLSON

Seconds after David Vetter was born, in 1971, he was placed in a specially designed crib. Plastic encased it—only sterilized items and filtered, germ-free air could enter. Vetter had severe combined immune deficiency (SCID). His immune system was so severely crippled that any infection could become life threatening. Doctors hoped that they'd develop a cure for his disease within a few years; they were unsuccessful. As the boy grew, he lived in larger and larger plastic bubbles, cut off from any direct physical contact with other people. Vetter died at the age of 12, known to the world as the “boy in the bubble.”

These days, isolating children in a bubble is almost never used as a therapy for this rare disease.

“The cost of the bubble and the emotional toll, it's just so extraordinary, that's not usually an option,” says Lisa Borghesi, assistant professor of immunology.

Some children can be cured with a bone marrow transplant—which is particularly successful if performed in the first three-and-a-half months of life. But only about 30 percent of SCID patients can find a matching bone marrow donor, and, among those who receive transplants, not all survive or have successful grafts. Children who don't receive a transplant often die before the age of 2.

Experimental gene therapy has yielded mixed results for a decade. In 2003, French researcher Alain Fischer administered gene therapy to 10 children whose SCID was caused by a single genetic defect. The treatment cured them of the disease—but three of the children developed cancer as a result

of the therapy. Even so, many researchers regard gene therapy as having promise as a cure for SCID.

But gene therapy depends on knowing which defective gene is causing the disease. Although several genetic mutations linked to SCID have been discovered, in 20 to 30 percent of cases, it's not known exactly what causes the disease. In these mysterious cases, we at least know that the cause has to do with B cells, which are vital to immune function. And research on B cells conducted by Pitt's Borghesi is paving the way toward answers.

B cells are created and developed in the bone marrow. When they mature, they exit the marrow and circulate throughout the body. Once a B cell circulates, it releases thousands of Y-shaped antibodies. Those thousands of antibodies are unlike the antibodies released from any other B cell in the body. The twin tips of the Y are the distinctive part of the antibody. That's where the antibody's receptors, which enable it to detect and grab hold of an invading particle, are found.

At any given time, millions of different *types* of antibodies circulate in our bodies. We have antibodies that would recognize SARS, avian flu, Ebola. Our bodies' strategy is to randomly produce millions of flavors of antibodies, so that no matter what the invading particle is, there will be a corresponding antibody whose receptors will recognize and latch onto surface proteins on the particle.

Those distinctive Y-tip sections make the immune response work. They are created by a process known as recombination, which takes place inside the nucleus of the B cell. Through recombination, the cell chops up

and reshuffles hundreds of tiny gene segments to form myriad patterns on a portion of the genome. Although changes to genes in any other cells might have grave consequences—like disease and death—these changes inside the B cells are essential to create the diversity of antibodies needed to defend our bodies against a wide array of invaders. Some people with SCID cannot make many antibodies because their recombination process is somehow blocked.

“Recombination is unique to the immune cells,” Borghesi says. “The Human Genome Project showed that the body has between 20,000 to 25,000 genes, yet we have millions and millions of different antibodies. So it's not possible that each gene encodes an antibody, because we don't have enough genes. But through recombination, the body takes the three genes that code [for antibodies] and turns them into millions of protein receptors.”

In the *Journal of Experimental Medicine* in 2004, Borghesi and her colleagues identified the stretch of B-cell DNA where the recombination process begins.

More recently she has learned that mice without a certain protein (transcription factor E47) show a 90 percent decrease in recombination activity, indicating this protein plays an important role in the process, yet there must be another factor at work as well.

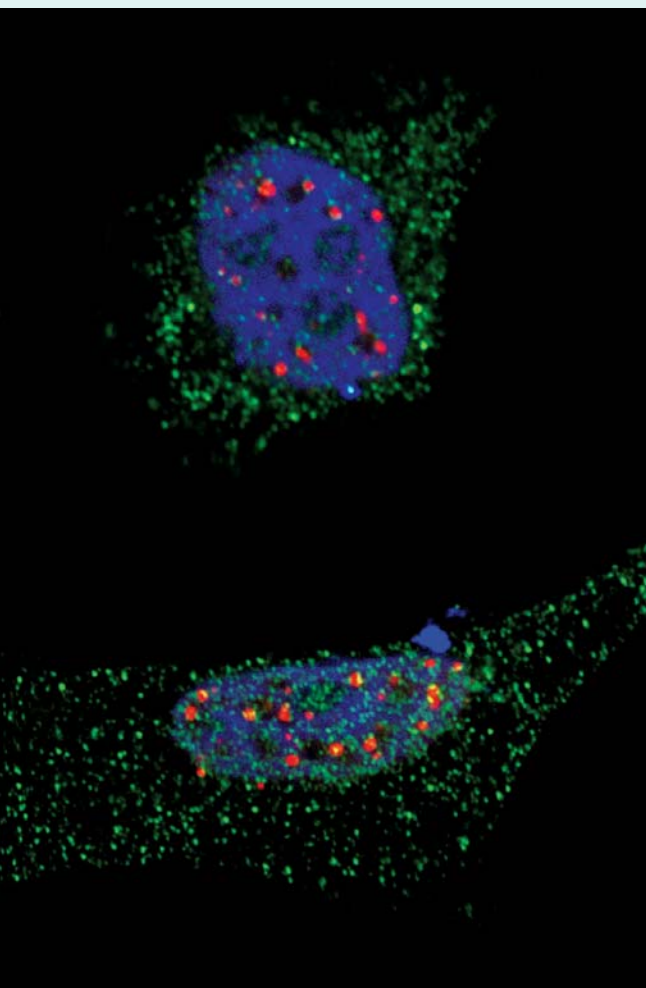
“Once we know the specific factors that turn recombination on and off in the mouse model, we'll look in patients and see if they have those factors.

“With gene therapy, there may be an opportunity to correct that defect if they don't,” says Borghesi. ■

HOW CELLS FLAG DOWN THE BIG GUNS

AND WHY RADIATION ISN'T ALWAYS
AN EFFECTIVE CANCER THERAPY

BY LEAH KAUFFMAN



COURTESY B. RAJASEKARAN

By revealing the secret lives of mismatch-repair proteins (shown here in intranuclear depots known as PML bodies), Rajasekaran has, among other things, told scientists why radiation and chemotherapy aren't effective against certain cancers.

Cells make mistakes. And who could blame them? According to the Human Genome Project, which mapped the minutiae of our DNA, there are perhaps 25,000 genes within each of our cells. As cells copy that huge volume of information in preparation for division, there's bound to be a clerical error or two.

Fortunately, there's a rigorous copy editor at work in most of us. As cells progress through the cycle of DNA replication and cell division, several checks assure that DNA is faithfully reproduced. Protein proofreaders scan lengths of DNA, checking for errors and omissions, alerting other proteins to drop in to make repairs. And if the DNA damage is too severe to be fixed, defective cells are discarded entirely.

"Proteins that are involved in these responses are actually genome protectors," says Pitt molecular biologist Baskaran Rajasekaran. So there's a lot of interest in how the process works—or doesn't. When these proteins stumble, cells with mutated DNA continue to replicate. By picking apart the subtleties of this protective process, Rajasekaran has revealed why radiation therapy is not always effective in warding off certain cancers.

For all our complexity, our 25,000 genes are made of just four molecules bound together in a specific way: adenine to thymine, cytosine to guanine. And for all the immutability of this pattern, sometimes

cells have been known to muddle things, say by joining an adenine to a guanine. Lucky for us, aptly named mismatch-repair proteins can catch these errors and correct them. Rajasekaran, who is an associate professor of molecular genetics and biochemistry in the School of Medicine, is interested in fully illuminating the function of these proteins.

Mismatch repair, though important, has been thought of as junior copy-editing stuff for proteins, like correcting punctuation. But in a 2003 *Nature Genetics* paper, Rajasekaran and colleagues described another, even more vital role for these proteins: They point out broken DNA to proteins equipped to bring cell replication to a halt and destroy the defective cell.

Those cell-killing heavies can't recognize DNA damage on their own. As Rajasekaran sees it, because the mismatch-repair protein has to look for damage anyway, it may as well hang out a flag to alert the big guns. (Although the current model of cell cycle proteins gives just one function to each kind of protein, "in reality this is not the case," Rajasekaran says. "It's much more complicated than that.") The newer model being built by him and others describes a myriad of functions for a protein, depending on which molecules it communicates with at which point in the cell cycle. These proteins are, in fact, jacks of many trades.)

Rajasekaran's findings explain why cell lines from certain inherited forms of cancer are resistant to radiation therapy. In garden-variety cancer cells, treatments like chemotherapy and radiation aim to jump-start DNA quality control, rendering cancer cells defective enough that they'll be marked for destruction. But in some forms of colorectal and endometrial cancer, the crucial mismatch-repair protein pennant is missing, so cells ravaged by radiation continue to chug through the cell cycle and divide: "They don't even know that they're damaged," says Rajasekaran. When the mismatch-repair protein is restored, news of damage is correctly communicated down the line, and the cell replication cycle functions as it should: Irradiated cancer cells cease to replicate, arrested just before their chromosomes separate. While this approach is still a long way off, Rajasekaran imagines one day dosing radiation-resistant cancer cells with the missing protein prior to therapy, so that chemo- and radiation therapies can do their work. ■

SO SOON?

WHY PRETERM BIRTHS ARE HIGHER
AMONG AFRICAN AMERICANS
BY DOTTIE HORN

When it's time to be born, the fetus usually emits a chemical communiqué, signaling to the mother's body, *Let me out*. At the receipt of this signal, the mother's immune system goes into action. It activates white blood cells in the uterus and cervix and liberates agents that bring about inflammation. This immune response ultimately makes the uterus contract and prepares the cervix for birth. Inflammation drives labor.

But in some cases, the inflammatory process starts too soon. Twelve percent of babies in the United States—nearly 500,000 each year—are born prematurely. Pitt research suggests that African American women may be genetically predisposed to premature labor because of subtle variations in their immune systems.

Hyagriv Simhan, assistant professor of obstetrics, gynecology, and reproductive sciences in the School of Medicine, conducted a study focused on a polymorphism in the gene for IL6, which often plays a role as an inflammatory protein. A polymorphism is a small peculiarity in a gene that typically is found in 1 to 10 percent of the population. We all have polymorphisms of one kind or another—thousands of them, in fact. As it turns out, people who carry this IL6 variant produce less of the natural inflammatory agent than people with the more typical version of the gene. (Other research has found that solid organ transplant recipients with this variant are less likely to reject an organ. Less IL6 seems to translate into a less robust immune response.)

Simhan found that women with the IL6 variant have a much lower risk of premature

delivery. He also discovered that African American women are less likely than Caucasian women to have the protective polymorphism. This helps explain why preterm birth is almost twice as common among African American women than among Caucasian women.

So how can the medical community help these women and their babies?

For the most part, efforts to prevent preterm birth have focused on mothers during the 11th hour—when they've already begun to go into labor—usually by administering uterus-paralyzing drugs. Now research is taking some impressive baby steps in a new direction. Scientists like Simhan, an MD who received his MS in clinical research from Pitt in 2001, are attempting to identify women at risk of early delivery so doctors can intervene before labor starts.

"We've explored using the body's own anti-inflammatory products as candidate pharmacologic agents," he says.

By this summer, he will begin testing these natural immune suppressors in rodent models of preterm birth. ■



RACIAL DISPARITIES LINKED TO INFLAMMATION

Are Blacks genetically predisposed to inflammation-associated conditions? It appears so. A recent study by Roberta Ness, Pitt's chair of epidemiology in the Graduate School of Public Health as well as professor of medicine, offers more clues to racial health disparities. In line with the findings of Pitt's Hyagriv Simhan (see "So Soon?"), she's concluded that the answers seem to lie in genetic polymorphisms, slight variants encoded in DNA. Ness found that African American women are more likely than Caucasian women to have polymorphisms that result in pronounced immune and inflammatory responses.

Says Ness, "African Americans suffer disproportionately from a variety of diseases, and many of those diseases are thought to be at least partially mediated by inflammation—heart disease, diabetes, preterm delivery, transplant rejection, autoimmune diseases [where the body goes into hyper-immune mode].

"Everything we found suggested that Blacks had a more revved up inflammatory response on the basis of genetics."

But such genetic variants are only part of the story.

"Genes don't act in a vacuum. Environment plays a huge role and probably plays a greater role, frankly, than genes do in predicting racial disparities," says Ness. "I don't think genetics is destiny. We need to think about how to alter the environment in such a way that this predisposition doesn't get triggered." —DH