Residents gather at the site of a bomb attack in Kirkuk, north of Baghdad, Iraq, in June. At least 65 people were killed when a truck packed with explosives detonated outside a mosque.
**BLASTS TO THE BRAIN**

**THE NEW WARTIME EPIDEMIC**

**BY REID R. FRAZIER**

If World War I brought shell shock, and the Vietnam War introduced the world to post-traumatic stress disorder (PTSD), the wars in Iraq and Afghanistan are etching a new entry in the lexicon of battlefield medicine: blast injury. There have been more than 80,000 detonations of improvised explosive devices in Iraq in the past six years, accounting for more than 60 percent of American and coalition-force casualties and the deaths of thousands of Iraqi civilians.

Typically, injuries in the “blast zone” are numerous and simultaneous—the blast can shred internal organs with shrapnel or shear off a limb. Among the most vulnerable areas in a blast is the brain. The brain can be confused, deformed, pierced, and damaged from secondary trauma such as ischemia, edema, and hemorrhage.

Armor, helmets, and better battlefield medicine have saved thousands of soldiers in these conflicts. (The killed-to-injured ratio in Iraq and Afghanistan is 1-to-10, compared to 1-to-2.5 for all other American wars.) But survivors are often severely injured. Soldiers with traumatic brain injury (TBI) induced by a blast suffer from headaches, dizziness, vertigo, gaze instability, and motion intolerance. Complicating care for these soldiers is the fact that blast TBI shares symptoms with PTSD. Military doctors and researchers are now trying to sort through the chaotic pathogenesis of this new, war-borne disease.

A team of University of Pittsburgh researchers led by Patrick Kochanek—an MD, director of the Safar Center for Resuscitation Research, and professor of critical care medicine, pediatrics, and anesthesiology—is helping the Department of Defense in this effort.

Kochanek and colleagues have found that blast TBI is quite different from “civilian” TBI, like the kind typically sustained in a car crash. “Say your head hits a windshield: You have this big, focal contusion. It’s a big focal area of badness that will ultimately be lost,” Kochanek says. “In blast TBI, the area of injury is far more diffuse.”

In studying the neuropathology of blast, Pitt researchers have found that, unlike its civilian cousin, blast TBI appears far more damaging to the synapses, axons, and dendrites of the brain—its white matter connective tissues and cables. These are the lines through which the electrochemical signaling of neurotransmission occurs.

“We’re getting an idea of how different this is from the conventional [civilian injury] model,” Kochanek says.

One culprit in the prevalence of axonal injury seems to be the increase in calcium signaling that occurs during blast TBI. Researchers have found that calcium pours through ion channels in injured neurons, releasing proteases suspected of cleaving proteins in the axons. (The prevalence of these cleaved proteins could serve as a biomarker for TBI on the battlefield.) So Safar researchers are looking at molecules known to moderate the calcium pathway—namely the immuno-suppressants cyclosporine and tacrolimus (the now-ubiquitous antirejection drugs tested in organ transplant patients at Pitt). Other team members are researching drugs that target the mitochondria, which are sensitive to “excitatory” calcium signaling.

Blast TBI commonly occurs alongside other injuries—penetrating wounds, traumatically amputated limbs, hemorrhage. This polytrauma only deepens the severity of brain injury. (In civilian TBI, polytrauma doubles morbidity and mortality rates.) Blood loss is a big factor in exacerbating the brain damage. When the body loses about 30 percent of its blood volume, as in a severe orthopaedic injury like a lost limb, hemorrhagic shock ensues. Blood vessels constrict throughout the body; blood pressure drops off a cliff. This is by design—the body tries to avoid bleeding out and conserves resources for the heart and brain. But when it’s injured, the brain needs blood—with its neuroprotective proteins and molecules—more than ever.

“It’s as if the brain is going through a 100-meter sprint, and you’re depriving it of oxygen,” says Kochanek.

Pumping too much blood into the body can lead to edema, a swelling of the tissues. So the Pitt team is testing blood substitutes that might be able to “thread the needle” by providing neuroprotective molecules without overloading the tissues with fluid. One possible approach is polynitroxylated pegylated hemoglobin (PNPH)—a blood substitute that has both antioxidant and expansive properties. Preliminary research shows PNPH, unlike standard hemoglobin, does not kill neurons in culture. (The team is also looking at a recombinant hemoglobin that was developed in E. coli and at a Carnegie Mellon University laboratory.) Induced mild hypothermia, an approach developed at Pitt, could also hold promise.

The nature of blast means that treating it will be complicated, says C. Edward Dixon, professor of neurosurgery, director of Pitt’s Brain Trauma Research Center, and a neurotrauma expert working on the project. “You may want to look at several types of treatments,” says Dixon. “There isn’t likely to be a silver bullet.”
When the results of an HIV-prevention study were released at an international conference in Montreal last February, the excitement in the room was palpable. Even though, at just 30 percent efficacy, the findings weren’t statistically significant, this was no small victory. It was the first time any medical product had been shown to reduce the incidence of HIV in women.

“It was quite intoxicating,” says Sharon Hillier of that electric day at the Conference on Retroviruses and Opportunistic Infections. “A colleague told me, ‘The first air flight was just a few feet off the ground. You have to take that first short flight before you can soar across oceans.’”

The study, dubbed HPTN-035, tested the efficacy of topical microbicide gels in preventing HIV infection. Two gels were tested against a placebo gel and against a no-gel control in more than 3,000 women in Southern Africa and the United States. The gel that was most effective was PRO 2000, which hinders HIV from attaching to target cells in the genital tract.

HPTN-035 was conducted by the Microbicide Trials Network, an international collaboration headed by Hillier and headquartered at Pitt. (Hillier, a PhD, is also a University of Pittsburgh professor of obstetrics, gynecology, and reproductive sciences, as well as vice chair for faculty affairs and director of reproductive infectious disease research in the Division of Reproductive Infectious Diseases and Immunology.)

The National Institutes of Health founded the network in 2006 to bring together investigators in the United States, Africa, and India developing new HIV-prevention strategies. It’s an urgent need, says Hillier; although AIDS treatment has advanced significantly in recent years, treatment alone can’t change the trajectory of the epidemic, which now affects 33 million worldwide. For every two people who receive treatment for the disease, five more people become infected.

Further, the old “ABC’s” prevention mantra of the past decade—abstain, be faithful, and use condoms—isn’t enough, especially for women.

“Abstinence arguably isn’t really a choice for many women,” says Hillier. “And condom use is not something women can control.”

The network is now testing the safety of a microbicide gel in a population researchers have found to be one of the most vulnerable: pregnant women. Pitt’s Richard Beigi, assistant professor of obstetrics, gynecology, and reproductive sciences, leads this phase I study, which is the first of its kind.

The network designed VOICE (Vaginal and Oral Interventions to Control the Epidemic), the first study to ask these questions. It began enrolling participants at sites in Southern Africa in September.

The network is exploring several AIDS-treatment drugs that might also be used as preventative drugs, an approach known as pre-exposure prophylaxis. One such agent is tenofovir. Hillier’s co-PI at the Microbicide Trials Network, Ian McGowan—Pitt professor of medicine in the Division of Gastroenterology, Hepatology, and Nutrition with a joint appointment in the Department of Obstetrics, Gynecology, and Reproductive Sciences—helped develop the drug.

McGowan is now working on two phase I trials of tenofovir’s safety, acceptability, and ability to be absorbed for use in anal sex, where the risk of HIV infection is 20 times higher than in vaginal sex. Rectal-microbicide research has long been underfunded, but has finally been embraced for its potential to combat the epidemic, McGowan reports. They’re trying two methods: tenofovir topical gels and tablets taken orally.

In July, Hillier received the Thomas Parran Award from the American Sexually Transmitted Diseases Association, a lifetime achievement award honoring her work in prevention and treatment of sexually transmitted infections. It’s a fitting tribute; the award was named after Thomas Parran Jr. (1892–1968), who was the first dean of Pitt’s Graduate School of Public Health. He, too, believed in the power of prevention and labored to bring to light a disease people would rather not think about: His focus was syphilis.

Nearly a century later, the story of AIDS is similar, Hillier notes. It’s a devastating disease that this country spends a fortune treating, though we neglect the prevention side. But Hillier and friends are hoping to help change that.

“What we’re hearing from colleagues and from study participants around the world is that this has given them hope,” she says.
Fifteen years ago, Steven Shapiro made a breakthrough in emphysema. He and his research team isolated and cloned an enzyme called macrophage elastase, or MMP-12, and went on to prove that it causes the deadly disease that chews up the elastic fibers of the lungs, at least in mice. Smokers, who gasp for air once their lungs turn baggy, have a high concentration of macrophage elastase, and mice that lack this enzyme are protected from smoking-induced emphysema.

Then five years ago, Shapiro, the Jack D. Myers Professor and chair of the Department of Medicine at the University of Pittsburgh, and Pitt’s McGarry Houghton, assistant professor of medicine, were conducting another lung study and made a startling discovery about macrophage elastase: The same enzyme that can be deadly also fights bacterial infections in the lungs and likely elsewhere in the body.

It was another eureka moment for Shapiro, as thrilling as his first discovery.

“We know the bad things it does. But Mother Nature is too smart to make things that are only going to hurt us,” says Shapiro. “They have to have some physiological function too.”

Shapiro and Houghton, who are both MDs, recently published their results in Nature. They are hoping their findings may lead to a potent new weapon against antibiotic-resistant “superbugs.” Collaborating with a structural biologist, they’ve begun working on a new antimicrobial drug.

“While our focus has been on understanding the biology of disease,” Shapiro says, “it sure would be nice to see something that came out of our research that actually helped patients.”

Their research is particularly relevant because of the proliferation of superbugs resistant to antibiotics, raising the fear that we could revert to the days prior to penicillin when routine infections could be life-threatening.

“Organisms are getting smarter and more resistant to antibiotics,” Shapiro says. “These superbugs are scary. We need fresh ideas to fight infections.”

MMP-12 is found in the macrophage, a cell that attacks invading bacteria. Houghton and Shapiro believe that the amino acid sequence of the antimicrobial portion of MMP-12, located in the tail of the enzyme, is unique in nature.

They tested the antimicrobial properties of MMP-12 on laboratory mice; half of the mice were genetically altered so that they had no macrophage elastase. The mice that lacked the enzyme hunched over and died when exposed to bacteria. The control mice with the enzyme thrived.

Shapiro and Houghton need to conduct more research to see whether the antimicrobial part of the enzyme would be safe for humans. They are also investigating whether macrophage elastase stops tumor cells from proliferating, as early evidence suggests.

The two researchers made their discovery while testing a mistaken theory regarding complications that can occur after bone marrow transplants.

But some of the best scientific discoveries come from mistakes, notes Shapiro: “If you are going to stick to your story, you are never going to get anywhere.”

Shapiro is so devoted to pulmonary research that he once started smoking for three months to serve as his own control group for an experiment. He stopped puffing after the widely watched experiment. (Many of his colleagues thought the effort was crazy.) Still, he remains devoted to studying emphysema and looking for treatments for other aspects of chronic obstructive pulmonary disease, or COPD. The disease is the fourth-leading cause of death in our country and is expected to be the third-leading cause of death in the world by 2020, he says. Although the incidence of smoking has gone down slightly, it has not yet been reflected in a lower incidence of COPD.

“I would love for everyone to stop smoking today,” Shapiro says. “But you would still have a whole generation of diseased lungs.”