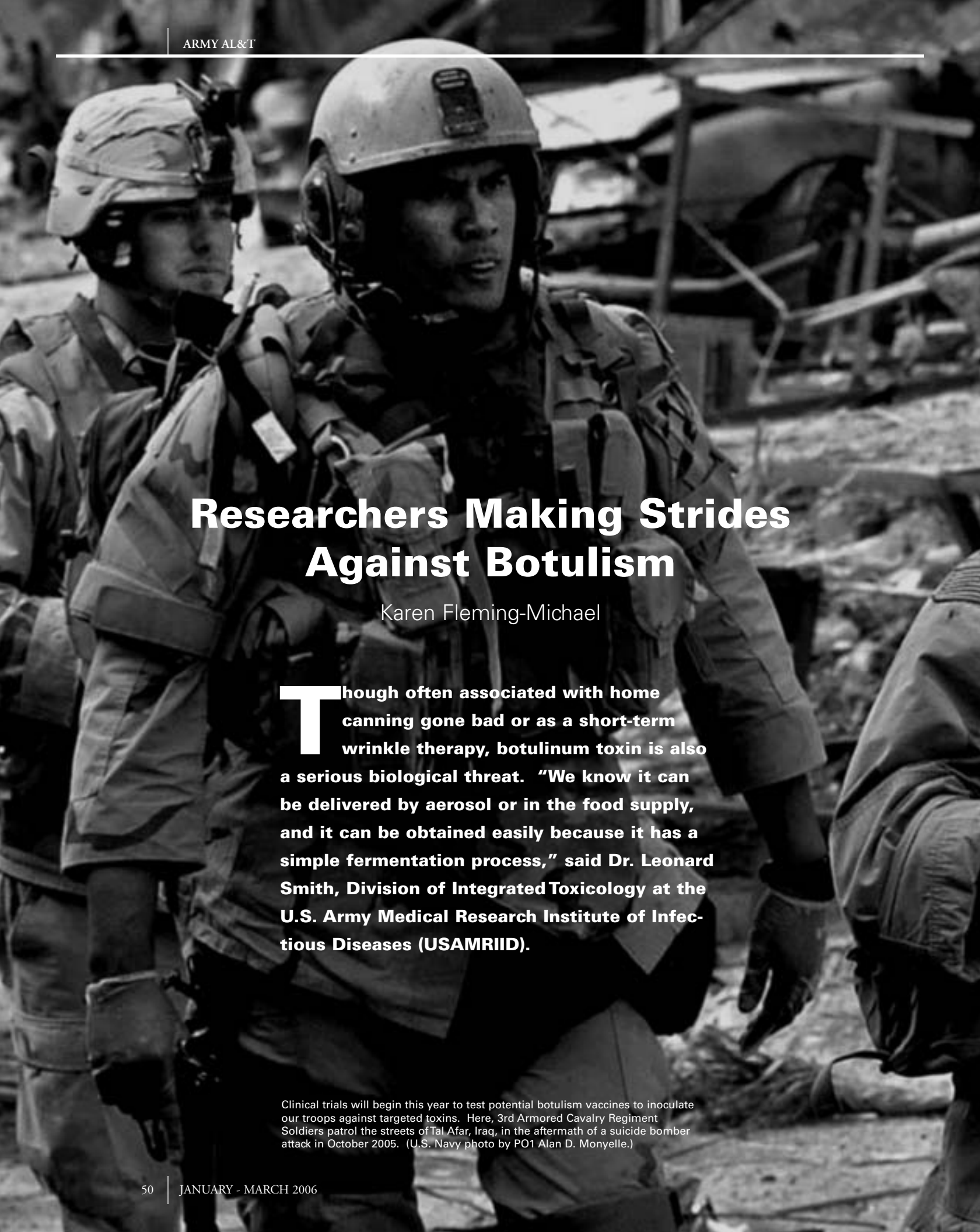

Researchers Making Strides Against Botulism

Karen Fleming-Michael

Though often associated with home canning gone bad or as a short-term wrinkle therapy, botulinum toxin is also a serious biological threat. "We know it can be delivered by aerosol or in the food supply, and it can be obtained easily because it has a simple fermentation process," said Dr. Leonard Smith, Division of Integrated Toxicology at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID).

Clinical trials will begin this year to test potential botulism vaccines to inoculate our troops against targeted toxins. Here, 3rd Armored Cavalry Regiment Soldiers patrol the streets of Tal Afar, Iraq, in the aftermath of a suicide bomber attack in October 2005. (U.S. Navy photo by PO1 Alan D. Monyelle.)

For decades, researchers at USAMRIID have led the way in finding, discovering and developing vaccines, treatments and diagnostic devices for the toxin that can kill or paralyze its victims. The full effect of the poison's power was demonstrated in November 2004 when media reports said a Florida doctor used a research formulation of the toxin as a substitute for the cosmetic product, Botox, that sent him and three others to hospitals where they ended up on respirators.

When the botulinum toxin acts, Smith said, clinical signs and symptoms of botulism first occur in the head region and progressively work their way south.

"First the cranial nerves are hit. Your eyelids get droopy, you can't swallow and then you can't breathe," he said. "You end up suffocating, so that's why people end up on respirators."

There are at least seven distinct toxin forms and they're designated types A through G. Though the forms are structurally similar, they're immunologically distinct, which means antibodies for one type will not protect against another type, Smith remarked. He, along with most of the people working on vaccines to prevent botulism at USAMRIID, received a toxoid vaccine that's presumably effective against five of the seven (A through E) serotypes.

The pentavalent toxoid vaccine was never licensed by the Food and Drug Administration (FDA). In fact, Smith persuaded some co-workers not to take the vaccine because they planned to work at the institute for only a few years.

"There is a growing list of clinical indications — such as spasticity and movement disorders, headache and pain, autonomic diseases, gastrointestinal and genitourinary maladies, and even cerebral palsy — that have been effectively treated using botulinum toxin," he continued. "Vaccination against the toxin would make those treatments ineffective, so I don't want them to run the risk that they'll need those treatments later in life and won't get help from them."

Beginning in the early 1990s, USAMRIID scientists started work on recombinant vaccine versions and have made one for each of the seven serotypes. The candidates for serotypes A and B transitioned to the Joint Vaccine Acquisition Program (JVAP) for advanced development in 1999 and have now been examined in an initial safety trial. USAMRIID continues to develop recombinant vaccines against types C, D, E, F and G toxins with support from the Defense Threat Reduction Agency and the National Institute of Allergy and Infectious Diseases (NIAID).

The safety trial, also called a Phase 1 trial, for the AB vaccine is being conducted by DVC LLC, JVAP's prime systems contractor responsible for developing and licensing the bivalent vaccine with the FDA. The Phase 1 trial's objective is to evaluate the safety of the vaccine in a small population of volunteers and to choose one or two

Although U.S. medical sources report that botulism toxins have never been used in warfare scenarios or bioterrorism events, the potential is there. Vaccines are being developed to protect Soldiers who may be exposed to the toxins in the future. (U.S. Army photo by SPC Chris Foster.)

doses to examine in the Phase 2 clinical trial. The clinical trial, based at the University of Kentucky, is being managed by Covalent Group, a clinical research organization based in Wayne, PA. To date, 44 volunteers have received vaccinations.

"The objective of the Phase 2 trial, which starts in 2006, is to select the vaccination schedule that ensures a fast and durable immune response because those things are obviously important to Soldiers," said Kathy Berst, the botulism vaccine manager for JVAP. "If they're going to deploy, they need a vaccine that will protect them as soon as possible."

Smith and his team, in collaboration with Dr. James Marks of the University of California in San Francisco, are also working on treatments that use human monoclonal antibodies to lessen the toxin's effects, so if a person is exposed to the botulinum toxin, they might not have an extended stay on a ventilator. The Centers for Disease Control and Prevention currently has a licensed antitoxin for serotypes A, B and E.

"The antitoxin neutralizes and clears the toxin from the circulatory system, but there's nothing to help the damaged nerve cells, so recovery can take months," Smith emphasized. Given a choice between treating botulism with an antitoxin or preventing it entirely, USAMRIID researcher Dr. Mark Poli prefers a vaccine. "Therapeutics are great, but they assume you're going to be sick. A vaccine means you never get sick, and that's the best of all worlds," he explained.

Having a device that can tell if a Soldier has been exposed to a biological agent, like botulism, is what Poli has been working on with a United Kingdom-based company called Akubio. "If you can put a detector out in the field that says a bot exposure has occurred before Soldiers start coming down with bot in the medical tents, lives can be saved," he said. "With bot, there's a window of opportunity for treatment ... primarily before symptoms show up. Once the symptoms show up, it is much more difficult to treat and save the exposed Soldier."

The device is still early in its development and uses acoustic sensing to detect a broad range of toxins, bacteria and viruses. "It actually listens for molecular interactions," Poli explained. Akubio employs an acoustic approach to detect agents in what can be a very simple device. The system uses a quartz crystal that has an electrical current running through it so it

vibrates millions of times each second. The crystal is electronically sampled to detect resonating sound waves. An antibody or a piece of DNA is attached to the crystal, and when a solution containing a biological agent such as a toxin, virus or bacteria is run over the vibrating crystal, the agent will attach to the antibody on the crystal.

Changes in resonance are immediately detected and indicate the presence of an agent. "As the energy is acoustic, you can hear it if you have the right listening device. This device is very smart and can detect more than one signal at once," Poli remarked. "We'll know what is present because the antibodies we put on the crystal are specific for it (agent)."

For example, put an antibody for Ebola on the crystal, and if the user hears resonance at the right frequency, it has to be Ebola. The approach, now in beta stage development, is termed and trademarked as *resonant acoustic profiling*. "Today, you can see the binding event by looking at the change in the resonant properties of the crystal," Poli continued. "Next year we expect to use additional properties to validate what we have found in each case."

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"We could potentially have a single chip that covers the top five bioterror agents and have a general alarm chip that says there's something there," said Matt Cooper, chief scientist at Akubio. "This approach could be extended to an ID chip with multiple sensors 'A to Z' with attached antibodies or DNA corresponding to toxins and pathogens '1-26.'"

This year, NIAID gave Akubio a 4-year grant for $3 million for further work on the technology. As the firm continues refining prototypes in Cambridge, England, USAMRIID will test their effectiveness in Frederick, MD. Akubio plans to broaden its research program during the grant period by adding further sounds that can be detected by its device giving even greater real-time insights to the infectious pathogens.

"The ultimate goal is to develop a hand-held detector that can run more than 1,000 samples on a rechargeable battery and doesn't need complicated software or computers," Poli reflected.

"We hope we get to the point where we have a functioning instrument and we know how to develop the chips, look at the signals and develop the assays, at which point we can show the Army we have something useful," Poli theorized. "There's a lot of work that goes between 'Here's a machine that can generate a signal,' and 'Here's a machine that can work in a real world at the concentrations that we need it to work and in the matrices that we need it to work in.'"

"Botulism," Smith added, "continues to be a concern at USAMRIID. Though the toxin has never been used in a warfare scenario or in a bioterrorism event, the Japanese cult that released sarin gas in the Tokyo subway in 1995 admitted to failed attempts in trying to use botulism as an aerosol. The intent was there," Smith concluded. The medical community is working hard on possible solutions.

An acoustic device that uses sound to detect a broad range of toxins, bacteria and viruses is being developed by Akubio, a United Kingdom-based company. (Photo courtesy of Akubio.)

KAREN FLEMING-MICHAEL is a Public Affairs Officer with the U.S. Army Medical Research and Materiel Command. She has a B.A. in English literature from the University of Maryland and an M.S. in public administration from Auburn University. She has worked as an editor and public affairs specialist for 16 years.