IN THE FIGHT AGAINST BIOTERRORISM, SURVIVAL TACTICS START IN THE LAB

CONSIDERING WORST-CASE BIOTERRORIST SCENARIOS SUCH AS PLAGUE, ANTHRAX AND SMALLPOX, AS WELL AS "OLD-FASHIONED" RADIOACTIVE DIRTY BOMBS, EXPERTS AT WFUBMC ARE RESEARCHING THE BEST IMMUNO-DEFENSE AND TREATMENT STRATEGIES.

BY ROBERT CONN

IN EUROPE AND THE UNITED KINGDOM, it's common to see paintings from the Middle Ages depicting effects of plague: the dead lying in streets, bodies stacked like cordwood in oxcarts. The plague wiped out nearly half the population of Europe and swept through London at least 12 times in the 16th Century.

Could it happen again?

Picture a plastic squeeze bottle containing aerosolized plague in the hands of bioterrorists. A few squeezes in a crowded football stadium, or in a jammed subway car, or in endless security lines in a major airport, and the plague could begin spreading fast.

"If you breathe in the plague bacteria, and you are not immune to it, you have 48 hours to get antibiotic therapy," says Steven B. Mizel, Ph.D., professor of microbiology and immunology. "If you don't, the likelihood is that you will be dead in another 48 hours."

The worst form is pneumonic plague, in the lungs. When an infected person coughs, the *Yersinia pestis* bacteria are carried in air droplets that are inhaled by others. It's a scary enough scenario that special federal research dollars have been set aside to seek answers.

Some of those special biodefense funds from the National Institutes of Health, $9.2 million over five years, have Medical Center scientists racing along three parallel tracks to develop an effective vaccine against plague. Exciting results already have emerged.

The same eight-member multidisciplinary team is also trying to develop a better smallpox vaccine. "Our charge was to create vaccines that are safe and fully protective against plague and smallpox," Mizel said. Smallpox is a deadly disease that has been virtually eradicated worldwide and is now supposed to be only in "safe" laboratories. The *vaccinia* vaccine used in the eradication campaign has cardiovascular and other side effects, and routine use of that vaccine has ceased.

Another group led by Al Claiborne, Ph.D., in biochemistry is using the techniques of structural biology to search for vulnerabilities in the anthrax bacterium, which was used as a bioterrorism weapon in the aftermath of 9/11. The inhaled form killed five of 11 infected people in 2001 following attacks through the mail.

The projects are part of a growing national defense focus at the School of Medicine which now totals nearly $4.6 million a year, with some 20 projects. Funding comes from the National Institutes of Health (NIH), the Department of Defense (especially DARPA — the Defense Advanced Research Projects Agency), the Department of Energy and the Department of Homeland Security.

Plague still occurs naturally in the United States, especially in the Four Corners area where Arizona, New Mexico, Utah and Colorado meet.

In May, Mizel reported at a national conference in Chicago that when mice immunized with a new combination vaccine were challenged with a lethal dose of *Yersinia pestis,* the immunized mice survived, "but the control mice succumbed in three days. We can put in 150 lethal doses of plague and these animals walk around like nothing happened."

The vaccine, developed here, includes a protein taken from the plague bacteria combined with a protein called flagellin, an adjuvant that multiplies the effectiveness of the vaccine several hundred thousand times. Flagellin is taken from bacterial flagella, the whip-like appendages that bacteria use to move around.

"We have also established that flagellin is an effective adjuvant in monkeys immunized with flagellin and *Yersinia pestis* antigen. The immunized monkeys show no undesired effects from the vaccine," Mizel said.

The immunized monkeys were not challenged directly with plague because the school does not have the high containment facility in which to run such a test. But other tests indicated the monkeys had the appropriate immune response. "It worked as well if not better in the monkeys than it did in the mice."

Therefore, Mizel said, it is highly likely that the combination will work in people.

How does it work? Mizel explained that there are two functional components of the immune system, innate immunity and adaptive immunity. "The link between innate and adaptive immunity is

AL CLAIBORNE, PH.D., JAMIE WALLEN AND CARLEITTA PAIGE (RIGHT TO LEFT) SEARCH FOR VULNERABILITIES IN ANTHRAX BACTERIUM.

DR. MARTHA ALEXANDER-MILLER AND GRADUATE STUDENT SHARMILA PEJAWAR ANALYZE THE MATURATION OF DENDRITIC CELLS FOLLOWING INFECTION WITH *VACCINIA* VIRUS.

crucial for any vaccine to work," he said.

Cells have receptors on their surfaces that recognize the presence of an invading bacteria's flagella and alert the rest of the immune system. Flagellin has the same effect. That alert process is innate immunity. The plague protein in the vaccine functions as an antigen, and the body produces antibodies to that antigen. That is adaptive immunity.

Mizel said he is trying to arrange to have the monkeys challenged elsewhere. Meantime, the team is in the process of injecting serum that contains the antibodies taken from the monkeys into mice — which produces "passive" immunization. These mice will be tested to see if they survive a challenge with *pestis.*

Mizel said they were also trying to simplify the combined vaccine into a single protein that contains both the plague antigen and the flagellin.

And he hopes soon to start clinical trials with flagellin — first to test for safety. "For me to get a clinical trial done is something that a scientist only dreams about," Mizel said, describing all the steps from his first recognition of flagellin's potency. "It would be an incredible experience to have carried something from an idea all the way to demonstration in humans. To be able to do that is extraordinarily exciting and satisfying."

But using the "naked" protein taken from the plague and injecting it into a subject along with flagellin is just one approach to inducing protective production of plague antibodies. Another approach is using a virus that doesn't cause human disease to "carry" the plague antigen into the body, which the scientists call a vaccine vector.

Douglas S. Lyles, Ph.D., chairman of the Department of Biochemistry, is testing a variant of vesicular stomatitis virus (VSV), while Griffith D. Parks, Ph.D., a virologist, is testing a variant of simian virus 5 (SV5). "We will be able to compare these different vaccination strategies to see what the advantages and disadvantages of each one are," said Lyles.

"What we do to make a vaccine strain of VSV is to introduce the gene for the proteins of plague or smallpox into the genetic information of the virus so that when it is used to infect animals or people, the virus will make these proteins and that will induce immunity against plague or smallpox," said Lyles.

Parks noted that when the wild-type SV5 virus invades a cell, it quickly shuts down the cell's defenses. "The wild-type virus stays in the infected cell and just keeps spewing out virus particles. What that tells you is the wild-type SV5 virus has a really potent mechanism to shut off host response."

When the SV5 variant invades similar cells, the host cell quickly senses it has been infected and eliminates the virus. The cell then will sense the plague protein, identify it as foreign, and initiate the making of antibodies against the plague.

But there are helpful processes that are part of the host defense. "When your body senses it is infected by a virus, it makes cytokines," Parks said. One of the most important cytokines is interferon, which limits the spread of the invading virus, promotes the antibody response, and calls in cytotoxic T cells to mop up the virus.

The wild-type SV5 and wild-type VSV block the interferon response, which is why they can invade successfully, he said, while neither variant can block interferon production, which makes them safer. In fact, the variant form of SV5 makes interferon and promotes the production of antibodies.

Lyles pointed out that the vaccines that result are not likely to be used on everybody. "These vaccines probably will be used on a more limited basis to vaccinate people that are exposed in the event of an attack."

The development of a new vaccine for smallpox is more complex than the plague vaccine, because three proteins for smallpox must be incorporated, along with flagellin. "We have to use these multiple proteins because there are two forms of the smallpox virus," said Mizel. "Based on our work with plague, we are hopeful and confident this will work as well."

Martha Alexander-Miller, Ph.D., associate professor of microbiology and immunology, is trying to understand what happens in initiating a respiratory tract infection, whether from plague, smallpox or another agent. The lungs are constantly bombarded with "foreign" entities, some dangerous, some not.

She has discovered, "The dose of virus that is lethal when given to mice through the intranasal route is much smaller than what is lethal when given through other routes" such as an injection in the abdomen. In addition, at high virus doses, the number of disease-fighting T cells declines rather than increases.

She found that high doses of virus caused a catastrophic loss of T cells that went well beyond the T cells that had been activated. "It looks like there is a generalized loss of lymphocytes — T cells and B cells — in these animals as a result of this virus infection," Alexander-Miller said. "This likely makes it harder to fight a subsequent infection."

She is also investigating dendritic cells, "the major cell type that drives the generation of a T cell response." Some invading viruses — such as the *vaccinia* virus she has been studying, prevent the dendritic cells from activating T cells, which could slow the body's immune response.

One goal: "You'd like to have a therapeutic that promotes the optimal expansion and retention of antiviral cells and prevents nonspecific loss of cells that could potentially put people in a generally immunosuppressed state," Alexander-Miller said.

Meantime, the structural biologists have shown that *Bacillus anthracis,* the causative agent of anthrax, along with a limited number of other pathogenic bacteria, uses a different biological process than most other bacteria, and for that matter, most other life forms including mammals and people.

They have shown that a vitamin B5 derivative known as Coenzyme A and its enzyme, Coenzyme A disulfide reductase, abbreviated as CoADR, play a crucial role in the life cycle of anthrax. But anthrax is missing a similar cofactor called glutathione (and its enzyme, glutathione reductase), which is found in most other bacteria, animals and people.

That means that scientists could develop therapeutic agents that target the CoADR processes without fear of disrupting human life processes. Their focus is on the anthrax spore, which germinates and becomes infectious in macrophages, white blood cells that ordinarily fight off disease.

"A key aspect of anthrax spore biology concerns the germination process through which the dormant spore becomes a reproductive, disease-causing bacterium," said Claiborne, co-director of Wake Forest's Center for Structural Biology. "Spore germination and outgrowth are fundamental to proliferation."

The CoADR function is also important in anthrax spore formation, he said. Spores are dormant "in conditions of adverse temperature, dehydration and without nutrition for years," but when the conditions are right, the spore germinates, and active bacteria emerge.

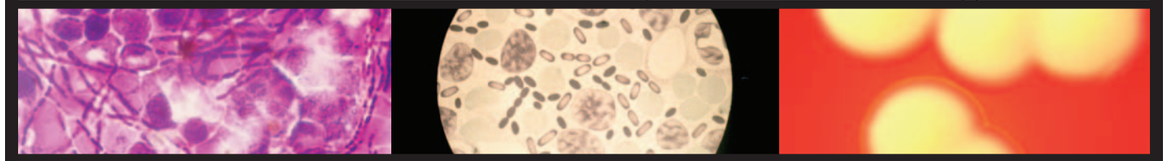
Another bacterium, *Staphylococcus aureus,* which causes many hospital-based infections, also uses a CoADR function. When Claiborne's group compared the already known genome sequences for both staph and anthrax, "we identified two genes in the anthrax chromosome that were closely related to *Staphylococcus aureus* CoADR."

He said a number of other priority pathogens, including plague, have similar proteins.

The Structural Biology researchers have determined the structure of the anthrax CoADR. Members of the group are now developing mutant strains of a noninfectious anthrax form that contain a disrupted copy of the bacteria's CoADR, and they plan on studying the resulting effect on germination.

Claiborne added, "If the process of germination from an ingested spore is inhibited, that would be a very desirable outcome."

ANTHRAX *Bacillus anthracis* PLAGUE *Yersinia pestis* STAPH *Staphylococcus aureus*



DEALING WITH A DIRTY BOMB

PLAGUE, SMALLPOX AND ANTHRAX are just part of the threat from terrorists. Another threat is from a so-called dirty bomb — a conventional explosive that is packaged with radioactive material that scatters when the bomb goes off. The bomb kills directly, but the radioactive material — and the contamination — also can kill.

Ralph B. "Monty" Leonard, M.D., Ph.D., an emergency physician with a pre-medical school background in atomic energy and experience working at several national laboratories, found "there were no protocols or information on how to deal with patients who are contaminated with radioactive materials."

So, 25 years ago, he got together with the people at the Radiation Emergency Assistance Center/Training Site (REAC/TS), an organization within the Department of Energy, and wrote a radiation accident protocol, "a detailed way of dealing with a patient who has come to the emergency department who either is contaminated with radiation or might be."

"Some of the people who went to Chernobyl (a nuclear power plant in the Soviet Union that blew up) actually took that article with them," Leonard said.

A new threat is the dirty bomb. "The original 1980 article was really aimed at one or two contaminated patients presenting themselves at the emergency department," Leonard said. "That is a completely different story than if a dirty radioactive device was set off and you had thousands of people converging on your hospital. You have to have a whole new strategy."

The revised protocol is now on the REAC/TS's Web site, part of the official federal government recommendations on dealing with dirty bombs. — ROBERT CONN