Taking the sting out of the anthrax vaccine

Staff Sergeant Michael Murphy, of the Boone, Iowa, National Guard Reserve, is ready to do his duty when it comes time for members of his unit to get the next of six shots given over an 18-month period for anthrax vaccination. Although he is not entirely happy about it, he told the *JCI*, "I really don't have another choice other than get out, and I'm not prepared to do that." He said he simply hopes he doesn't have another adverse reaction. Soon after his third shot, Murphy suffered leg cramps caused by blood clots. "We can't really pinpoint the real reason I came down with this," he noted, "other than it's just a coincidence that I happened to get the shots and then came down with this. But I think the military, or whoever is building this vaccination, should follow up a little more with the people who had issues with it — to see if it did in fact have a reason for it, or was a problem at all, or why it happened."

While Murphy remains equivocal about the relationship between his health issues and the anthrax vaccine that is currently mandatory for military personnel considered to be at risk for biological weapons offensives, others, such as retired US Air Force Reserve pilot Lieutenant Colonel Jay Lacklen, who was stationed at Dover, Delaware, are adamant that the vaccine is to blame for myriad problems, primarily autoimmune responses that resulted in symptoms of extreme vertigo, intense muscle and joint pain, or mental impairments and ailments. Lacklen said that the occurrence of one of "those three [types of symptoms] almost immediately after the series of shots started at Dover caused 40% of our reserve pilots to leave the unit rather than take the shot."

A great deal of controversy has surrounded the use of the anthrax vaccine, including speculation as to whether it is the cause of Gulf War syndrome. There have been numerous accusations and investigations, and while the vaccine is still approved for mandatory military use, the uproar regarding its overall safety continues (see http://www.milvacs.org and http://www.anthrax.osd.mil for more information). The vaccine at the center of this storm uses a cell-free filtrate — a mix of dead bacteria as opposed to live bacteria — to stimulate the appropriate immune response. Only BioPort Corp. manufactures it; that fact, along with its history of having repeatedly failed FDA inspections, has further stirred the furor over its mandatory use among military personnel.

Robert Belshe believes that a lot of fascinating work is now going on in the development of non-needle vaccines.

Now come the next generation of anthrax vaccines, which are based on the use of recombinant protective antigen (rPA), a main component of anthrax exotoxin, to stimulate the protective immune response. Such a vaccine is already in phase II clinical trials.

Interest, however, in developing a needle-free anthrax vaccine is high. Two recently studied vaccines, one applied via a skin patch (1) and one that is inhaled (presented at the 228th National Meeting of the American Chemical Society in August; see http://oasys2.confex.com/acs/228nm/techprogram/P784204.HTM for abstract), show promise in their ability to protect against anthrax in preliminary animal studies.

The major advantage of needle-free vaccines is that they directly target places where most infections initiate.

"The [development of] non-needle vaccines is a whole fascinating field," Robert Belshe told the *JCI*. Belshe, who is the director of the Center for Vaccine Development at Saint Louis University and helped develop FluMist, a flu vaccine nasal spray, highlighted the importance of making these vaccines pain-free, since a "substantial proportion of the adult population won't get their influenza shot because it's a needle and they're afraid of it." He noted that there were other issues that make the development of needle-free vaccines even more worthwhile. For example, "for developing nations, where they may be reusing needles or boiling needles, there is always the question, are you transmitting hepatitis or AIDS or something through a parietal injection?"

Gary Matyas, of the Walter Reed Army Institute of Research, who coauthored the paper on the development of a skin-patch version of the anthrax vaccine (1), added that a patch system "is much easier to administer. You don't need much training to give it, and we should be able to deploy it into the field."

Belshe and Matyas both pointed out, however, that the major advantage of needle-free vaccines is that they directly target places where most infections initiate. Noel Harvey, director of Advanced Drug Delivery at BD Technologies and head of the group developing the inhaled anthrax vaccine, agreed, explaining that "the development of vaccines [that] can be rubbed onto the skin or placed into the nasal mucosa or the pulmonary mucosa is really undertaken, not so much to avoid using a syringe, but to actually get a mucosal response, in the case of intranasal or pulmonary delivery, or to access the Langerhans cells and dendritic cell–type precursors in the epidermis, in the case of delivery through the skin."

Both the skin-patch version and the inhaled version of the anthrax vaccine do use rPA to stimulate a protective response but are in preliminary animal-testing stages.

Matyas and his colleagues at Walter Reed, in a joint venture with IOMAI

Corp., have tested the anthrax patch on mice, immunizing them at 0-, 2-, and 4-week intervals with a gauze pad soaked with rPA and differing amounts of heat-labile enterotoxin (HLT) from *E. coli* as an adjuvant. At every level of HLT, the mice showed 100% protection against anthrax (Sterne strain) challenge. Matyas told the *JCI* that although this work was done using HLT, "part of the research effort that we are doing here is to look at other adjuvants," and it is of note that rPA alone, without any adjuvant, also afforded 100% protection (1).

In the inhaled-vaccine studies at BD Technologies, which are being conducted in collaboration with the US Army Medical Research Institute of Infectious Disease, the nasal cavity is targeted. The vaccine formulation utilizes rPA with a mucosal adherent called chitosan. "We are very early in this research and we are very encouraged with the findings of protection in rabbits with a relatively simple powder formulation of recombinant protective antigen," Harvey said. "In those formulations with no other additives that could be termed adjuvants [beside the mucoadhesive and CpG], we did achieve protection of 100%."

The finding that rPA alone might stimulate a strong protective response may be good news for many in the military, since a great deal of the controversy over the safety of the current BioPort anthrax vaccine centers on the effect of the adjuvant. While the BioPort vaccine uses aluminum hydroxide — which is standard in many US vaccines — as an adjuvant, it has also been found to contain trace amounts of squalene, an adjuvant that is not approved for vaccine use in the US but is used in some European vaccines. Squalene is known to cause autoimmune reactions when injected into animals. Although many have discounted the trace amounts as too small to cause the types of reaction some have experienced, this finding has created even more concern over the use of the current vaccine.

Progress of these non-needle vaccines from preliminary stages to approval for human use, however, is many years off. Standard vaccine approval requires extensive clinical testing after animal testing is complete. Belshe explained that an appropriate dosage for the antigen in the vaccine is determined through a series of tests in small animals. "And then you go through a process of evaluating the vaccines in humans. Typically, young healthy adult volunteers are given the first dose of vaccine, and if it's ultimately going to be a childhood vaccine, then you move gradually into younger and younger populations. Or if it's targeted for older folks, you gradually work into an older population. You do this stepwise in small numbers of persons so that you minimize risk and yet achieve reasonable milestones of understanding of what's going on."

Gary Matyas envisions that the skin-patch vaccine, packaged like a Band-Aid, could be easily deployed where needed.

A vaccine for anthrax, or any other deadly infectious agent, obviously cannot ever be tested in a challenge study in humans. Harvey stated that, for the anthrax vaccine, "the general next steps are to do dose-titration steps, to see if there is an optimal dose range to capitalize on, then move into larger studies with protective correlates of man, like the rabbit model we have used, then into higher primates to assure ourselves that we are going to obtain protection and that the vaccine is safe and doesn't stimulate any undue responses or have any side reactions associated with it."

The vaccine would never be used in the general population, as are those for measles and smallpox, but would be used only in at-risk populations.

There would, however, be no real certainty of the vaccine being protective in humans. Matyas did note, though, that there are ways to obtain a sense of the protective capability of a vaccine. "You can assay it for toxin neutralization titers. At least in rabbit models, neutralization titers correlate with protection. In humans one would have to make that assumption. But of course that is not proven."

The vaccine would never be used in the general population, as are those for measles and smallpox, but would be used only in at-risk populations. Currently, that means people in the military, such as Michael Murphy and Jay Lacklen. While Murphy might be resigned to receiving such a vaccine and accepting its risks, he said that he "would want to know what some of the effects of it could be. And I would maybe want to know if someone has a family history of something that could affect them by taking it." Lacklen remains suspicious, given what he has seen at Dover Air Force Base, and he finds the military's answers to his questions about the presence of squalene in the current vaccine unconvincing. He believes that military personnel are being used as test subjects. Lacklen told the *JCI*, "I don't think anthrax is that potent a weapon on the battlefield. I think the entire anthrax hype is to run the vaccine." He added that he would not be convinced that a new vaccine was safe unless its chain of custody had been closely monitored and it was then tested for the presence of squalene.

Protection from infectious toxins for these men and women is important, but from their standpoint, assurance of the safety of the vaccine that should protect them seems only fair.

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1. Matyas, G.R., et al. 2004. Needle-free skin patch vaccination method for anthrax. *Infect. Immun.* **72**:1181–1183.