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Keeping the lock on smallpox

David B Weiner

Since the eradication of natural smallpox infection, research efforts have waned and the vaccine is now outdated. Two studies revitalize efforts to create a new smallpox vaccine. One study moves vaccine production from animals into tissue culture; the other examines duration of immunity in already vaccinated individuals (pages 1125–1139 and 1131–1137).

Greek mythology tells the story of Pandora, who lived with her husband, Epimetheus, in a time when the world was free of evils and plagues. One day, Pandora discovered a beautiful box with a sign that said, "Do not open!" But Pandora's curiosity got the better of her. She lifted the lid, and all the troubles and plagues of the world immediately escaped. Of all the imaginary terrors that could have come out of Pandora's box, it is hard to imagine that any could be more evil than the very real orthopoxvirus smallpox.

From 1850 to its eradication in 1979, almost 1 billion people worldwide succumbed to smallpox infection[1,2]. After 11 September 2001, fears of deliberate infection caused the world to look at smallpox in a new light. It remains possible that stocks of the virus exist outside of their sanctioned deep-freezes in Russia and the US[1–4]. Smallpox is a serious terror threat: it can be produced in large quantities, is stable enough for storage and transportation, has a 30% mortality rate in exposed, nonvaccinated individuals and has been known to spread from a single infected individual to 10–20 or more people[2,5].

Several vaccine-related issues need to be addressed to ensure public safety. These include the need for a modern alternative to the live animal–produced stock and to determine immune correlates relevant for the twenty-first century in order to test new, safer vaccine candidates. In this issue, Hammarlund *et al.*[6] and Weltzin *et al.*[7] move us considerably closer to these goals. Hammarlund *et al.* provide evidence that vaccine-induced immunity persists for many years and is, in some fashion, lifelong. Weltzin *et al.* reveal a new tissue culture method for producing smallpox vaccine that bypasses the methodology of the twentieth century, which required scraping the hides of cows infected with vaccinia virus (**Fig. 1**), an attenuated orthopox virus that generates cross-protective immunity against smallpox in humans.

Millions worldwide, and about 90% of individuals in the United States over the age of 35 (half the country's population), were vaccinated before the end of the mass vaccination campaigns. The status of their immunity against smallpox has been under debate[4,8–11]. Hammarlund *et al.* measured T-cell immunity against vaccinia virus in 306 vaccinees, up to 75 years after their last vaccination. Within the first 7 years after vaccination, $CD4^+$ and $CD8^+$ T-cell responses remained high and then declined slowly over decades. The decline in $CD4^+$ T cells, which facilitate immune expansion, occurred more slowly than the decline in $CD8^+$ T cells, which eradicate viral factories. Yet even between 41 and 75 years after vaccination, most vaccinees showed some $CD4^+$, and some had $CD8^+$ T-cell immunity. When they examined humoral immunity, Hammarlund *et al.* found that most subjects maintained stable antibody responses for up to 75 years after vaccination, suggesting essentially lifelong immunity. This conclusion dovetails with previous studies suggesting that antibody responses that neutralize the virus are associated with effective vaccination. In addition, there seems to be little immunological benefit to multiple vaccine boosts given early, suggesting that the first immunization 'protects' against subsequent vaccination.

The consequences of the waning of cellular immune responses observed by Hammarlund *et al.* require further study. Is the decrease in $CD8^+$ T-cell responses an early indication of vaccine failure, or might $CD4^+$ T-cell help permit a rapid expansion of $CD8^+$ immunity upon challenge and infection? Studying the usefulness of additional vaccination for people later in life and the expansion of their T-cell response is important, as is studying the effect of decreased T-cell immunity in the presence of persistent antibody in nonhuman primate challenge models. Perhaps heterologous prime-boost strategies, which would not

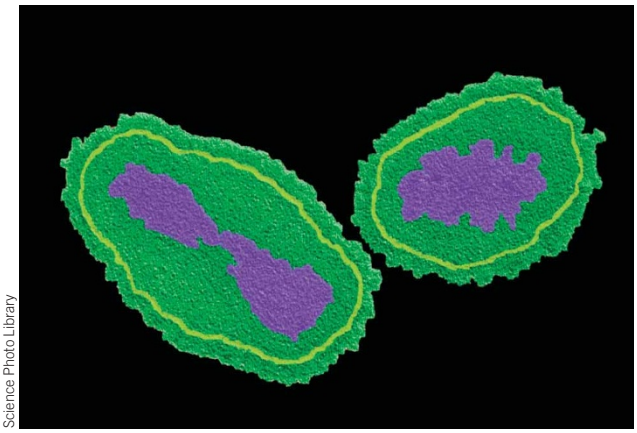

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Figure 1 Colored transmission electron micrograph of a section through vaccinia virus particles. The genetic cores (purple) are covered by membrane layers (green and yellow) taken from the host cell in which the virus replicated. This virus is the basis for the smallpox vaccine used in the twentieth century and investigated by Hammerlund *et al.* Weltzin *et al.* introduce a new vaccine using vaccinia adapted to tissue culture.

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be antibody limited, might be beneficial[12]. The persistent immune responses observed by Hammarlund *et al.* suggest that side effects of vaccination, such as eczema vaccinatum[13] should occur infrequently in revaccinated individuals. The study also suggests ways to determine correlates of protection in the absence of true smallpox challenge.

While the study by Hammarlund *et al.* indicates that most vaccinated individuals may retain some immunity against smallpox infection, Weltzin *et al.* introduce a first-generation replacement for those without immunity. This is an important accomplishment because the current vaccine stocks, even if diluted, would probably not fulfill the demands of unvaccinated individuals in the United States, let alone worldwide[5,8,9].

Weltzin *et al.* adapted the existing Dryvax vaccine, which is derived from the crossprotective vaccinia virus, to a human cell line for production in tissue culture. After animal studies, they chose one clone as the most promising vaccine candidate. In a small clinical study in humans, 100% subjects vaccinated with the new vaccine (ACAM-100) vs. 97% of Dryvax-vaccinated subjects exhibited the hallmark of vaccine take, a significant cutaneous reaction at the site of scarification. Overall, ACAM-100 induced a robust immune response, although there were differences between the two vaccines. For example Dryvax induced higher antibody titers while ACAM-100 vaccination seemed to result in stronger CD4 T cell responses. The vaccines had similar safety profiles, with each participant experiencing at least one mild to moderate adverse event.

Unfortunately, Weltzin *et al.* did not use the same assays as those used in the longitudinal studies of Hammarlund *et al.* In the future, such comparisons would increase confidence in this new vaccine. Challenge studies in nonhuman primate models are also warranted.

The safety profile of ACAM1000 seems similar to, and perhaps slightly better than, that of Dryvax. However, the investigators still observed a low rate of lethality in suckling mouse studies, as well as the induction of encephalitis in nonhuman primates, as is the case with Dryvax. In addition, the issue of cardiac toxicity, which has recently plagued vaccination efforts, needs to be clinically evaluated[14]. In fact, the mass vaccination of health-care workers and law-enforcement personnel has recently been put on hold because of safety issues.

Until proven safe, only when faced with the threat of a smallpox outbreak should such a vaccine be considered for mass vaccination campaigns. The ACAM1000 vaccine, rather than being a truly new approach, is a modern production strategy for an old vaccine. The goal of a new safe and effective smallpox vaccine has not yet been attained[15,16], but a suitable stopgap seems to be in place.

These two studies provide new technology as well as a suitable benchmark for the development of the next generation of vaccines. However, the reagents necessary for detailed and defined analyses of humoral and cellular responses in this effort are still lacking. A central repository for reagents to study agents of bioterrorism will significantly aid this effort. In addition, pharmaceutical approaches that could be useful in treating smallpox infection should be moved forward to testing in nonhuman primate studies as rapidly as possible. Such therapeutic agents will further alleviate vaccine dependence and provide an additional cushion of safety.

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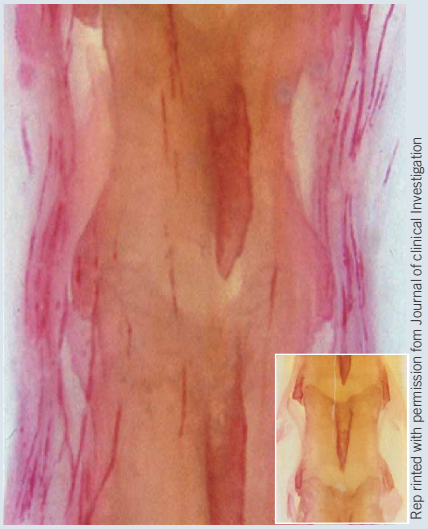
Counteracting calcification

Despite high levels of phosphate and calcium in the body, mineralization normally occurs only in bones and teeth. But when calcification afflicts other regions of the body, as occurs in diseases such as atherosclerosis and chronic renal failure, tissues can be severely damaged. What keeps these minerals in check? In the 1 August *Journal of Cell Biology* Schäfer *et al.* provide evidence that an abundant serum protein negatively regulates calcification. The investigators examined mice deficient in this protein, a cysteine protease named α_2-Heremans-Schmid glycoprotein (Ahsg, also known as fentuin-A). The mice were living on the edge. They appeared phenotypically normal but developed severe calcification of various organs while on a mineral- and vitamin D–rich diet. Shown here are calcified deposits in small vessels in muscles along vertebrae, from an Ahsg-deficient mouse fed the enriched diet for 4 months (inset, from wild-type mouse). The Ahsg-deficient mice, in combination with a genetic background sensitized to calcification, also developed severe calcification. Moreover, patients with calciphylaxis. a severe calcification disorder, had low serum Ahsg.The results jibe with previous *in vitro* studies showing that Ahsg inhibits formation and precipitation of basic calcium phosphate, a mineral central to bone formation.

Charlotte Schubert



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