



Biosafety Tips

Karen B. Byers

Dana Farber Cancer Institute, Boston, Massachusetts

Biosafety Tips brings you practical approaches to biosafety or “news you can use.” If you are looking for a useful and sensible solution to a biocontainment problem or perhaps a reference to help convince a skeptical researcher of the need for caution, this is the place to look. In this column I will share some biosafety insights for managing a variety of workplace situations. I welcome feedback or suggestions for future topics. Please e-mail any comments or suggestions to karen_byers@dfci.harvard.edu or to Co-Editor Barbara Johnson at barbara_johnson@verizon.net.

Biosafety Issues in Laboratory Experiments Using Vaccinia Viral Vectors

Vaccinia vectors allow the insertion of large (2 kb) genomic segments. The foreign DNA will not be integrated into the host cell, and expression of the foreign gene will be at relatively high levels (Kost, 2001). These advantages prompt molecular biologists to use viral vectors constructed from a variety of vaccinia virus strains, some of which are highly attenuated strains of the virus and others that are non-attenuated. Communicating the risks of working with vaccinia is a challenge for the biosafety professional.

Highly Attenuated Strains of Vaccinia

TROVAC (fowlpox) and ALVAC (canarypox) strains do not replicate in human cells, and NYVAC (derived from Copenhagen strain) replicates poorly in human cells. When working with these strains, CDC advises using Biosafety Level 1 (CDC, 2001).

MVA (Ankara) is listed as another highly attenuated strain; however, the containment recommended for it is Biosafety Level 2 (CDC, 2001). Smallpox vaccination is not recommended for work with TROVAC, ALVAC, NYVAC, or MVA (Ankara) because, in the event of an exposure, these viruses do not replicate and produce a clinical infection. However, vaccinia vectors made from highly attenuated strains are effective in presenting the genetic insert as an antigen in human cells, and this must be considered when conducting the recombinant risk assessment. In fact, recombinant vectors made from highly attenuated strains of vaccinia are used in human gene transfer trials (CDC, 2001).

Nonattenuated Strains of Vaccinia

Working safely with nonattenuated vaccinia strains [NYCBOH, Western Reserve (WR), Copenhagen, or Lister] requires a thorough understanding of the infectivity of these viruses, the benefits of smallpox vaccination, and the protective practices of Biosafety Level 2. In the United States, Dryvax[®] is the licensed smallpox vaccine; it is prepared from the New York City Board of Health (NYCBOH) strain of vaccinia virus. Smallpox vaccination is recommended for laboratory staff handling vaccinia or infected animals. Vaccination provides controlled percutaneous doses of approximately 2.5×10^5 PFU of NYCBOH, a well-characterized virus of low pathogenicity. This prompts a protective immune response in the event of an uncontrolled exposure incident involving a larger dose or a virus of higher or unknown pathogenicity. In addition, persons with pre-existing immunity to vaccinia *might* be protected against seroconversion to the foreign antigen ex-

pressed by a recombinant virus if inadvertently exposed (CDC, 2001). Researchers may be reluctant to receive smallpox vaccination because there are a number of medical contraindications to vaccination. In addition, precautions must be followed to prevent autoinoculation or dissemination of virus from the inoculation site. Discussing all of these issues improves the researchers' understanding of the vector system they plan to use.

Laboratory staff members who are unfamiliar with the constraints of working at Biosafety Level 2 may interpret the initial training session as "a lot of rules." To improve acceptance of the requirements and propel researchers to change from casual BSL-1 to stringent BSL-2 work practices, a discussion of laboratory-acquired infections is helpful. Current literature has several graphic descriptions of vaccinia virus laboratory-acquired infections, and these may be used to improve understanding of BSL-2 containment requirements and increase acceptance of smallpox vaccination.

Examples of Incidents

The list below summarizes nine published cases of laboratory-acquired infection with vaccinia virus.

- A postgraduate student was hospitalized with high fever, severe swelling, and inflammation of the face. He had recently had an eyebrow pierced, but bacterial cultures of the wound were negative. He also had a lesion on his finger. The student had been infecting mice with a thymidine kinase deletion mutant of the WR strain but did not sustain a needlestick or other known exposure. When a laboratory colleague visited the hospital, he noted that the lesion on the finger looked like vaccinia. The eyebrow wound was also infected with vaccinia, probably through autoinoculation. The infected individual had not been vaccinated (HSE, 2005).
- An individual working with WR strain, thymidine kinase deletion mutant, sought medical attention for a lesion on his finger. This person had received training in lab techniques and the risks of working with vaccinia, but he did not know the symptoms and did not know he was infected. The infected individual had not been vaccinated (HSE, 2005).
- An experienced researcher who worked with vac-

cinia for 10 years handled a cell culture with a high titer of recombinant virus (10^6). He did not have an exposure incident; however, he developed an infection in his fingers. In this case, the palms of his hands were mildly roughened from work in cold temperatures, but he had no underlying dermatological conditions. The recombinant virus had an immunomodulating insert which may affect leukocyte adhesion; this may have made the infection more serious. The researcher was vaccinated at 1 year and 12 years of age; the infection occurred at age 40 (Mempel, 2003).

- A woman with eczema noticed that wearing gloves exacerbated her dermatitis. Therefore, she did not wear gloves when handling vaccinia virus and became infected with the strain she was handling. She received medical attention twice for her infected fingers before hospitalization with the appropriate diagnosis. Fortunately, she recovered completely. She was not vaccinated (Loeb, 2003).
- A technician in a laboratory working with vaccinia received a minor cut from a coverslip. Twelve days later, a "pimple" developed at the site; she squeezed it and pus squirted onto her chin. One to 2 days later, a lesion appeared on her chin. The cut was not considered the exposure incident because of the length of time before the lesion appeared; it is assumed that the cut became contaminated later, followed by autoinoculation on the chin (Wlodaver, 2004).
- While injecting mice with an infectious vaccinia virus recombinant expressing the N gene of VSV, a small cut on the right ring finger was inoculated with a drop of virus. The individual washed his hands immediately. Five days later, the finger was swollen and reddened; a pock developed on day 10. The virus was not attenuated by insertional inactivation of the thymidine kinase gene. The infected individual had been immunized 30 years earlier. Testing revealed antibody to VSV proteins (Jones, 1986).
- A technician was manipulating five recombinant vaccinia viruses (rVV) expressing individual proteins from RSV. Fine needles held in the right hand were being used to transfer live virus to materials in the left hand. This procedure had been used for 13 months without incident, until one Friday afternoon a needle slipped and penetrated the glove over the

left thumb. The technician continued working and also inoculated her forefinger with a different rVV. A localized infection developed. The author felt the incident would have been more serious had she not been vaccinated just 2 years before it occurred (Openshaw, 1991).

- While harvesting purified virus, a technician sustained a needlestick. Six days later, pustules appeared on all of her fingers, and on the eighth day necrotic tissue from her fingers had to be surgically excised. The infected individual had not been vaccinated since childhood (Moussatche, 2003).

- A researcher sustained a splash of IHD-J strain of vaccinia on some abrasions on his hand. Three days later localized erythematous pruritic papules developed at the site of the splash. On day 6, he sought medical attention for localized vaccinia. He was afebrile and without adenopathy. His vaccination 7 months earlier had been a “take”; the lesions resolved without treatment (Rusnak, 2004).

Additional Incidents with a Related Pox Viral Vector (Raccoon Pox)

- A staff member was injecting mice with raccoon pox vector and, during disposal, sustained a needlestick with the used needle. The inoculum was estimated to be less 15 microliters of a 10^7 pfu/ml solution; however, she developed a localized infection (Rocke, 2004).

- A woman was bitten by her dog when she attempted to remove a sachet containing raccoon pox from her dog’s mouth. Raccoon pox-laden sachets are used to control rabies; poxviruses are so stable that they can be used in pellets distributed in the wild for rabies control (Rupprecht, 2001).

This extensive list of recent laboratory-associated infections should raise awareness of the infectivity of vaccinia and vaccinia vectors. VIG, or sterile immune globulin prepared from the plasma of vaccine recipients, is available for the treatment of adverse reactions to vaccinia vaccination or for high-risk exposure incidents. However, its use is not recommended for vaccinia keratitis (CDC, 2001); the importance of eye protection when handling these viruses should be obvious. The following points from the LAI reports may stimulate discussion about vaccinia virus and proper containment.

- CDC recommends vaccination for work with nonattenuated strains of vaccinia (CDC, 2001).

- Infections with this vaccinia may occur in the absence of a known exposure incident. Contact with contaminated surfaces is considered the probable source of infection (HSE, 2005; Mempel, 2003).

- Researchers should know the symptoms caused by infection with vaccinia (HSE, 2005; Loeb, 2003; Wlodaver, 2004), and should disclose their potential workplace exposures when seeking medical attention. Two of these cases were further complicated by autoinoculation (HSE, 2005; Wlodaver, 2004).

- Gloves should be worn; this simple practice would have prevented two laboratory-acquired infections (Loeb, 2003; Rusnak, 2004).

- Wherever possible, sharps should be eliminated from Biosafety Level 2 experiments. A needle should not be used to harvest purified virus (Moussache, 2003).

- LAIs have occurred with thymidine-kinase mutants of the WR strain (HSE, 2005).

- LAIs have occurred with recombinant viruses (Jones, 1986; Mempel, 2003; Openshaw, 1991; Rocke, 2004; Rupprecht, 2001).

- In two laboratory-acquired cases, the individuals developed an immune response to the genetic insert (Jones, 1986; Rocke, 2004). This should prompt a good discussion of the proposed experiment. What are the consequences of exposure to the genetic insert in the experiment undergoing review?

Occupational Health Considerations

Contraindications to Administration of Smallpox Vaccine

Are staff members aware of the other contraindications to receipt of vaccinia vaccine? This is important; clearly, anyone with one of these contraindications is at increased risk for a laboratory-acquired infection if an exposure incident occurs. The list of contraindications for administration of smallpox vaccine is reprinted below.

1. Eczema

“Because of the increased risk for eczema vaccinum, vaccinia vaccine should not be administered to persons with eczema of any degree, those with a

past history of eczema, those whose household contacts have active eczema, or whose household contacts have a history of eczema. Persons with other acute, chronic, or exfoliative skin conditions (e.g., atopic dermatitis, burns, impetigo, or varicella zoster) might also be at higher risk for eczema vaccination and should not be vaccinated until the condition resolves” (CDC, 2001).

2. Pregnancy

“Live-viral vaccines are contraindicated during pregnancy; therefore, vaccinia vaccine should not be administered to pregnant women for routine non-emergency indications. However, vaccinia vaccine is not known to cause congenital malformations. Although <50 cases of fetal vaccinia infection have been reported, vaccinia virus has been reported to cause fetal infection on rare occasions, almost always after primary vaccination of the mother. Cases have been reported as recently as 1978. When fetal vaccinia does occur, it usually results in stillbirth or death of the infant soon after delivery” (CDC, 2001).

3. Altered Immunocompetence

“Replication of vaccinia virus can be enhanced among persons with immunodeficiency diseases and among those with immunosuppression (e.g., as occurs with leukemia, lymphoma, generalized malignancy, solid organ transplantation, cellular or humoral immunity disorders, or therapy with alkylating agents, antimetabolites, radiation, or high-dose corticosteroid therapy [i.e., ≥ 2 mg/kg body weight or 20 mg/day of prednisone for ≥ 2 weeks]). Persons with immunosuppression also include hematopoietic stem cell transplant recipients who are <24 months posttransplant, and hematopoietic stem cell transplant recipients who are ≥ 24 months posttransplant but who have graft-versus-host disease or disease relapse. Persons with such conditions or whose household contacts have such conditions should not be administered vaccinia vaccine” (CDC, 2001).

4. Persons Infected with HIV

“Risk for severe complications after vaccinia vaccination for persons infected with HIV is unknown.

One case of severe generalized vaccinia has been reported involving an asymptomatic HIV-infected military recruit after the administration of multiple vaccines that included vaccinia vaccine. Additionally, a 1991 report indicated that two HIV-infected persons might have died of a progressive vaccinia-like illness after treatment with inactivated autologous lymphocytes infected with a recombinant HIV-vaccinia virus. No evidence exists that smallpox vaccination accelerates the progression of HIV-related disease. However, the degree of immunosuppression that would place an HIV-infected person at greater risk for adverse events is unknown. Because of this uncertainty, until additional information becomes available, not vaccinating persons (under routine non-emergency conditions) who have HIV infection is advisable” (CDC, 2001).

Adverse Events from Smallpox Vaccine

Apparently, it is an open question whether adverse cardiac events are associated with the use of the vaccine. The 2003 U.S. smallpox vaccination campaign reported suspected or probable myo- or pericarditis in 22 of 38,257 civilian vaccines and in 63 of 515,000 military vaccines (Upfal, 2004). No secondary transmissions from civilian vaccines occurred. There were 14 cases of inadvertent inoculation of contacts of military vaccines and two cases of ocular vaccinia (Smallpox Vaccine Adverse Events Coordinators, 2004).

After discussing the occupational health information with researchers, you should take this opportunity to refer them to the occupational medical services at your institution. My employer requires staff to visit occupational health services for screening and education on smallpox vaccination if they plan to work with nonattenuated vaccinia. Vaccination may be accepted or declined. However, staff members with medical conditions that would contraindicate smallpox vaccination are prohibited from working with nonattenuated vaccinia, since they would be at increased risk of infection if an exposure occurs.

References

- Centers for Disease Control and Prevention. (2003). Smallpox vaccine adverse events coordinators; National Immunization Program. Update: Adverse events following civilian smallpox vaccination—United States. *Morbidity and Mortality Weekly Report*, 53(05), 106-107. Available at www.cdc.gov/mmwr/preview/mmwrhtml/mm5234a4.htm.
- Centers for Disease Control and Prevention. (2001). Vaccinia (smallpox) vaccine recommendations of the Advisory Committee on Immunization Practices (ACIP). *Morbidity and Mortality Weekly Report*, 50, (RR10), 1-25. Available at www.cdc.gov/mmwr/preview/mmwrhtml/rr5010a1.htm.
- Health and Safety Executive Advisory Committee on Genetic Modification. (2005). Incidents—lessons to be learnt—Accidental infection with vaccinia virus. *Newsletter* 32. Available at www.hse.gov.uk/biosafety/gmo/acgm/acgm32/paper8.htm.
- Jones, L., Ristow, S., Yilma, T., & Moss, B. (1986). Accidental human vaccination with vaccinia virus expressing nucleoprotein gene. *Nature*, 319, 543.
- Kost, T., Condreay, P., & Mickelson, C. (2001). Biosafety and viral gene transfer vectors. In D. Fleming & D. Hunt (Eds.), *Biological safety principles and practices* (3rd ed.) (pp. 579-597). Washington, DC: ASM Press.
- Loeb, M., Zando, I., Orvidas, M. C., Bialachowski, A., Groves, D., & Mahoney, J. (2003). *Canada communicable disease report*, 29(15), 134-136. Available at www.phac-aspc.gc.ca/publicat/ccdr-rmtc/03vol29/dr2915eb.html.
- Mempel, M., Isa, G., Klugbauer, N., Meyer, H., Wildi, G., Ring, J., Hofmann, F., & Hofmann, H. (2003). Laboratory acquired infection with recombinant vaccinia virus containing an immunomodulating construct. *The Journal of Investigative Dermatology*, 120, 356-358.
- Moussatche, N., Tuyama M., Kato, S. E. M., Castro, P. V., Njaine, B., Peralta, J. M., Damasco, C. R. A., & Barroso, P. F. (2003). Accidental infection of laboratory worker with vaccinia virus. *Emerging Infectious Diseases*, 9(6), 724-726. Available at www.cdc.gov/ncidod/EID/vol9no6/02-0732.htm.
- Openshaw, P. J. M., Alwan, W. H., Cherrie, A. H., & Record, F. M. (1991). Accidental infection of laboratory worker with recombinant vaccinia virus. *Lancet*, 338, 459.
- Rocke, T. E., Dein, F. J., Fuchsberger, M., Fox, B. C., Stinchcomb, D. T., Osorio, J. E. (2004). Limited infection upon human exposure to a recombinant raccoon pox vaccine vector. *Vaccine*, 22, 2757-2760.
- Rupprecht, C. E., Blass, L., Smith, K., Orciari L. A., Niezgodna, M., Whitfield S. G., Gibbons R.V., Guerra, M., & Hanlon, C. A. (2001). Human infection due to recombinant vaccinia-rabies glycoprotein virus. *New England Journal of Medicine*, 345, 582-586.
- Rusnak, J. M., Kortepeter, M. G., Hawley, R. J., Anderson, A. O., Boudreau, E., & Fitzen, E. (2004). Risk of occupationally acquired illnesses from biological threat agents in unvaccinated laboratory workers. *Biosecure Bioterror*, 2(4), 281-293.
- Upfal, M. J., & Cinti, S. (2004) Adverse cardiac events after smallpox vaccination. *Emerging Infectious Diseases*, 10(5), 961-962. Available at www.cdc.gov/ncidod/EID/vol10no5/03-0967_04-0235.htm.
- Wlodaver, C. G., Palumbo, C. G., & Waner, J. L. (2004). Laboratory-acquired vaccinia infection. *Journal of Clinical Virology*, 29, 167-170.