

Minireview

Risks associated with vaccinia virus in the laboratory[☆]Adam MacNeil^{*}, Mary G. Reynolds, Inger K. Damon

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ARTICLE INFO

Article history:

Received 22 September 2008

Returned to author for revision

23 November 2008

Accepted 26 November 2008

Available online 31 December 2008

Keywords:

Vaccinia

Laboratory-acquired infection

Orthopoxvirus

Vaccination

Smallpox vaccine

ABSTRACT

Vaccinia virus (VACV) is used commonly in research laboratories. Non-highly attenuated strains of VACV are potentially pathogenic in humans, and VACV vaccination and biosafety level 2 facilities and protocols are currently recommended for vaccinated laboratory workers in the United States who handle non-highly attenuated strains of the virus. Despite this, laboratory-related VACV exposures continue to occur and a number of recent instances of VACV infection in non-vaccinated laboratory workers have been documented. We provide a discussion of the usage and risks associated with VACV in laboratory research.

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Background

The practice of immunizing with orthopoxviruses dates back over 200 years, in which the concept of vaccination was introduced by Edward Jenner, to protect against variola virus infection, the causative agent of smallpox. While it is not entirely clear what orthopoxvirus was first used for vaccination practices, vaccinia virus (VACV) was eventually (if not initially) used as the viral agent for smallpox vaccination, and its successful usage was largely responsible for the global eradication of smallpox. Similarly, a natural reservoir for VACV has yet to be identified and the origins of the virus are not known (Baxby, 1977); explanations for the origin of VACV include (i) the possibility that this was a zoonotic virus that has since gone extinct in nature, (ii) that VACV was artificially derived from another orthopoxvirus through serial passage during vaccine production, and (iii) that VACV may be a recombinant between variola and the original species of orthopoxvirus that was used for vaccination (Binns and Smith, 1992; Shchelkunov et al., 2005). Genetically, VACV is closely related to horsepoxvirus, however, the presence of gene disruptions in the horsepox genome, that are intact in the VACV genome, does not support the origin of VACV from horsepox (Tulman et al., 2006).

Vaccination with VACV provides long-lasting protection against a variety of orthopoxviruses, including variola and monkeypox viruses (Fine et al., 1988; Jezek et al., 1987, 1988). Among human vaccines,

VACV is unique in that it is delivered as a live, non-attenuated virus, by puncture of the skin overlying the deltoid with a bifurcated needle. Because of this, adverse events following vaccination are known to occur, particularly among those with high risk conditions, such as individuals with eczema, atopic dermatitis, or other skin conditions, individuals who are immunocompromised or pregnant, and those less than 1 year of age (Casey et al., 2006; Cono et al., 2003; Wharton et al., 2003). Secondary transmissions to close contacts of vaccinees can also occur. Out of 37,901 volunteers vaccinated under the US Department of Health and Human Services preparedness program, 722 nonserious adverse events and 100 serious events, including 85 hospitalizations, were reported (Casey et al., 2005). Similarly, in a study of laboratory workers receiving VACV vaccine, a wide variety of post-vaccination symptoms were identified, although most common symptoms tended to be relatively mild (Baggs et al., 2005). In August 2007, the Food and Drug Administration (FDA) licensed the new-generation ACAM2000 vaccine, which is now used in place of the Dryvax vaccine, in the United States (CDC, 2008) and clinical trials suggest similar safety, as well as efficacy (Greenberg and Kennedy, 2008).

VACV in laboratory research

VACV is commonly used in modern molecular biology research. Fig. 1 shows cumulative yearly numbers of publications (from National Library of Medicine's *Pubmed* database) with 'Vaccinia' in the title or abstract. While numbers of publications have increased slightly over the past decade, it is apparent that VACV usage has long been well-established in laboratory research. Consistent with this observation, VACV is used in the laboratory for a wide variety of purposes. As an example, we classified all abstracts, with 'Vaccinia' in the title or

[☆] The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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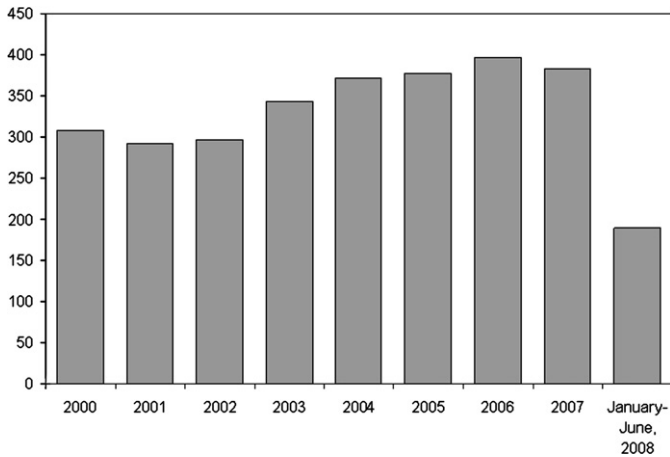


Fig. 1. Number of abstracts with 'Vaccinia' listed in the title or abstract, by year of publication (National Library of Medicine's *Pubmed* database).

abstract, with publication dates between January–June 2008, on the basis of the role of VACV in the research (Fig. 2). These include both studies involving VACV that are directly related to the understanding of orthopoxviruses, as well as usage of VACV as a functional tool for other areas of research.

In terms of research directly related to orthopoxviruses, the largest proportion of VACV-related studies fall in the area of basic virology (25% of abstracts). Other areas of research directly related to orthopoxviruses, that commonly involve VACV, include studies involving immune response to VACV infections, studies involving experimental orthopoxvirus vaccines, studies on anti-orthopoxvirus drugs or compounds, and studies related to public health issues surrounding orthopoxviruses.

As shown in Fig. 2, nearly half of all studies involving VACV are not related to orthopoxvirus research, but instead use VACV as a research tool. These types of studies can be broadly grouped into two areas: studies that use VACV as a protein expression or antigen delivery tool for basic immunology research (22% of abstracts) and studies that use VACV as a backbone for expression of foreign antigens for development of vaccines against unrelated agents (25% of abstracts).

VACV serves as an effective vector in the expression of foreign proteins for a variety of reasons. First, VACV infects a variety of cell types and results in high levels of protein expression. Additionally, viral replication occurs in the cytoplasm, and thus does not necessitate nuclear processing and transport of RNA. Finally, straight-forward, well-developed systems currently exist for the generation of recombinant VACV (Moss, 1996). Currently, most recombinant VACV strains are generated through insertional recombination in the viral thymidine kinase (TK) locus, resulting in a recombinant TK-minus viral strain (Chakrabarti et al., 1985; Mackett et al., 1984; Moss, 1996).

While the widespread use of TK-minus strains of VACV clearly demonstrate that this gene is nonessential for viral replication, studies do suggest that abrogation of TK function results in a modest attenuation in virulence. For instance, Buller et al. (1985) observed lower levels of infectivity and virulence of TK-minus strains of VACV in mice when delivered by intracerebral and intraperitoneal inoculations and similarly, Lee et al. (1992) observed a decrease in intracranial virulence in TK-minus strains of VACV. It should be noted, though, that both studies reported similar viral titers for wildtype and TK-minus strains on the skin, following intradermal inoculation (Buller et al., 1985; Lee et al., 1992). Additionally, human infections with TK minus strains of VACV following laboratory-related exposure have been well-documented (2008; Jones et al., 1986; Lewis et al., 2006; Mempel et al., 2003; Openshaw et al., 1991), indicating that recombinant TK-insertion strains of VACV are still pathogenic in humans.

A VACV strain is considered non-highly attenuated if the virus maintains the capacity to replicate productively in mammalian cells. Numerous non-highly attenuated strains of VACV currently exist. Of central importance to current laboratory research is the Western Reserve (WR) strain of VACV which was selected by serial intracerebral passage in mice, for neurotropic potential. Other strains used in the laboratory commonly are related to the New York City Board of Health (NYCBH) strain, from which the Dryvax and ACAM2000 vaccine strains, as well as VACV WR, were derived (ATCC, 2008; Wokatsch, 1972). In contrast, attenuated strains of VACV do not have the capacity to replicate in mammalian cells. Of note is the modified vaccinia Ankara (MVA) strain, which is able to infect and result in protein expression, but not replicate, in mammalian cells (Sutter and Moss, 1992), and may serve as a possible alternative in the future to the currently licensed VACV vaccine (Phelps et al., 2007).

Laboratory safety and VACV infections

Because non-highly attenuated strains of VACV are pathogenic in humans and handling virus in the laboratory presents a possible risk of infection, safety guidelines have been developed. As a vaccine, VACV has been shown to be highly efficacious in generating protective immune responses against both smallpox and monkeypox, and furthermore, studies indicate robust VACV-specific immune responses in humans following vaccination (Amanna et al., 2006). Therefore, pre-exposure vaccination with VACV is a primary intervention that can prevent or minimize the effects of accidental exposure in the laboratory. Consistent with this observation, the Advisory Committee on Immunization Practices (ACIP) recommends VACV vaccination for laboratory workers in the United States who handle cultures or animals contaminated or infected with non-highly attenuated VACV strains, at least every 10 years (Rotz et al., 2001).

In addition to vaccination, the usage of at least Biosafety Level 2 practices and facilities are currently recommended for the manipulation of viruses or animals infected with non-highly attenuated VACV strains (2007). This includes the usage of proper personal protective equipment, including gown, gloves, and eyewear protection when procedures have the risk of splash, proper laboratory facilities and safety equipment, proper decontamination of infectious material, and proper animal facilities (2007).

Despite the availability of vaccination and worker adherence to safety procedures, laboratory-acquired VACV infections do occur and

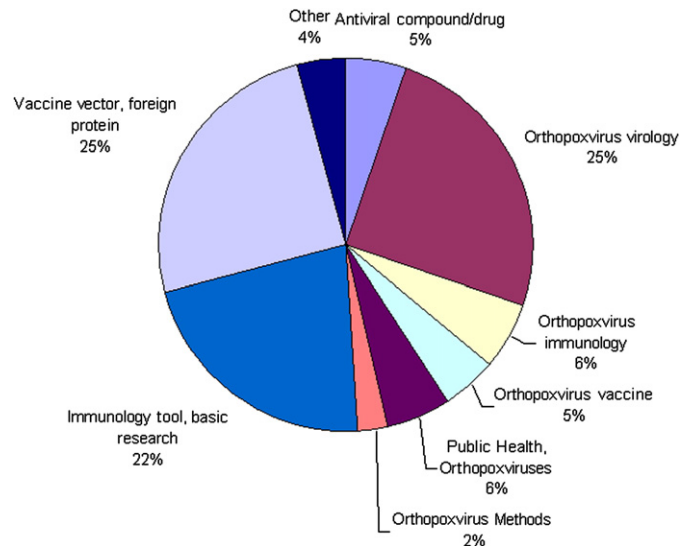


Fig. 2. Publications with 'Vaccinia' in the title or abstract, January–June 2008 (National Library of Medicine's *Pubmed* database). Review articles and articles not in English were excluded.

have been well documented in the literature. Inadvertent exposures have occurred through needlestick accidents or eye splash (2008; Jones et al., 1986; Lewis et al., 2006; Loeb et al., 2003; Mempel et al., 2003; Moussatche et al., 2003; Openshaw et al., 1991; Wlodaver et al., 2004). Laboratory-acquired VACV infections have commonly involved recombinant viruses, which express foreign proteins (2008; Jones et al., 1986; Lewis et al., 2006; Mempel et al., 2003; Openshaw et al., 1991) produced from non-highly attenuated strains, such as WR (2008; Jones et al., 1986; Lewis et al., 2006; Mempel et al., 2003; Moussatche et al., 2003; Openshaw et al., 1991).

There exists no formal surveillance system in place, within the United States, for instances of laboratory-related orthopoxvirus exposures or infections. However, the Poxvirus Team at the Centers for Disease Control and Prevention (CDC) has been contacted on a number of occasions in recent years regarding instances of laboratory-related orthopoxvirus exposures (Table 1). The majority of these instances have involved VACV (typically WR) harboring a foreign gene in the TK locus. Exposure to VACV most commonly occurred through accidental needlesticks or through eye splash accidents. In 6 recent instances, exposure resulted in VACV infection, and 4 of 6 infections resulted in subsequent hospitalization. Infections commonly involved fever and large focal areas of painful induration, erythema and severe swelling around the inoculation site. Hospitalizations occurred to assess the need for surgical or medical intervention. Five of the 6 cases involved infection of the hand or digits, and the remaining individual had ocular and facial involvement. None of the six infected laboratory workers had met the ACIP vaccination recommendation for working with non-highly attenuated VACV in the laboratory.

Discussion

VACV is commonly used in laboratory research and the occurrence of laboratory-related VACV infections has been documented previously (2008; Jones et al., 1986; Lewis et al., 2006; Loeb et al., 2003; Mempel et al., 2003; Moussatche et al., 2003; Openshaw et al., 1991; Wlodaver et al., 2004). In recent years, we have received a number of reports of laboratory-related VACV exposures and infections. Additionally, laboratory-acquired VACV infections commonly have been the result of TK-minus strains of virus. Because studies in mice have suggested that inactivation of the VACV TK locus results in decreased virulence (Buller et al., 1985; Lee et al., 1992), it may be perceived there is not a risk of infection associated with handling a TK-minus VACV strain; however, it is apparent that TK-minus strains of VACV do maintain pathogenic potential in humans.

Because we currently do not have an estimate on the number of laboratories, or researchers who are potentially exposed to non-highly

attenuated VACV strains, or the actual number laboratory-associated VACV infections that occur, it is not possible to accurately assess the overall risk of VACV infection, associated with laboratory work. However, the observation that a large number of exposures, and all recent infections, involve individuals who did not follow the ACIP recommendations for vaccination warrants consideration for a number of reasons. It is apparent that many researchers are hesitant to be vaccinated, because it is perceived that the risk of adverse events associated with vaccination is higher than the risk of working with VACV. While the risks associated with vaccination are thoroughly described to those considering vaccination, the benefits of receiving vaccination are often overlooked. There are a number of reasons that researchers should consider vaccination:

1. Vaccination involves controlled delivery of the virus to the skin overlying the deltoid. This is a region of the body that can easily tolerate swelling without compromising function or causing significant pain. Accidental infection on other parts of the body (e.g., hand or digit) can result in severe pain and swelling, and possible long-term sequelae. Furthermore, many would consider the cosmetic effects of a vaccination scar on the deltoid to be less objectionable than scarring on another part of the body, such as the hand, eye, or face, which are locations commonly associated with accidental infection.
2. Vaccination involves inoculation with a controlled dosage of a well-characterized virus strain. By contrast, laboratory-related exposures can result in the delivery of a high titer of virus, as well as delivery through an atypical route, such as deep injection or ocular inoculation. Furthermore, laboratory studies commonly involve recombinant VACV strains, which have the potential to result in altered viral virulence or artificially modulated immune response to the virus.
3. Adverse events associated with vaccination are generally mild and severe adverse events are rare. For instance, 0.22% of vaccinations administered under the US Department of Health and Human Services preparedness program resulted in hospitalization (Casey et al., 2005). By contrast, 4 of the 6 (66%) recent laboratory-acquired VACV infections reported to CDC resulted in hospitalization.
4. Although the risk of exposure can be minimized by handling the virus under proper laboratory conditions and using proper techniques (for instance, the usage of eye protection can prevent ocular exposure), it is often not possible to completely eliminate the risk of accidental exposure to VACV. Personal protective equipment cannot provide complete protection from needlestick accidents, which account for a large proportion of VACV exposures.

Table 1
Laboratory-related orthopoxvirus exposures reported to CDC, 2005–2008

Year	State	Virus (strain, if known)	Met ACIP vaccination?	Nature of accident	Result in infection?
2005	CA	Vaccinia	No	Eye splash	No
2005	FL	Vaccinia (rabbitpox)	Yes	Eye splash	No
2005 ^a	CT	Vaccinia (recombinant WR)	No	Needlestick	Yes (hospitalization)
2006 ^a	PA	Vaccinia (recombinant WR)	No	Needlestick	Yes
2006	CT	Vaccinia	Yes	Eye splash	No
2007 ^a	IA	Vaccinia (recombinant WR)	No	Needlestick	Yes
2007	NM	Vaccinia	Yes	Animal care facility	No
2007 ^a	MD	Vaccinia (recombinant WR)	No	Needlestick	No
2007 ^a	NH	Vaccinia (recombinant WR)	No	Needlestick	Yes (hospitalization)
2007	MA	Vaccinia (recombinant NYCBH)	No	Needlestick	Yes (hospitalization)
2007	MO	Monkeypox	Yes	Needlestick	No
2008	GA	Vaccinia	Yes	Animal care facility	No
2008	CA	Vaccinia (recombinant WR)	No	Eye splash	No
2008	NH	Vaccinia (recombinant WR)	No	Eye splash	No
2008	VA	Vaccinia (recombinant WR)	No	Unknown	Yes (hospitalization)
2008	FL	Vaccinia	Yes	Tube leakage	No

^a Detailed account of case provided elsewhere (2008).

- The possibility exists for inadvertent secondary transmission of VACV from an infected individual. Because of potential delays between laboratory-related exposure and recognition of symptoms, laboratory workers may put contacts, such as family members and healthcare workers, at risk for infection.

Conclusion

The eradication of smallpox through the administration of VACV vaccine is one of the greatest public health achievements in history. VACV remains an important virus today for laboratory-based research, in the study of virology, immunology, and in the development of novel vaccines. However, VACV is potentially pathogenic in humans, and laboratory-acquired infections continue to occur. Although adverse events have been associated with VACV vaccination, post-vaccination symptoms tend to be relatively mild, and the ACIP currently recommends VACV vaccination for laboratory workers who handle non-highly attenuated VACV strains. While the highly attenuated MVA vaccine is not currently licensed in the United States, laboratory workers will hopefully benefit from this vaccine in the future. The usage of proper safety measures, including administration of VACV vaccine, can minimize the risk of conducting research which involves this virus.

Acknowledgments

We thank our state health department colleagues, and the research institution officials and physicians, who assisted in investigation of the orthopoxvirus exposures and infections, described in [Table 1](#).

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