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The effects of post-exposure smallpox vaccination on clinical disease presentation: Addressing the data gaps between historical epidemiology and modern surrogate model data

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Abstract

Decades after public health interventions – including pre- and post-exposure vaccination – were used to eradicate smallpox, zoonotic orthopoxvirus outbreaks and the potential threat of a release of variola virus remain public health concerns. Routine prophylactic smallpox vaccination of the public ceased worldwide in 1980, and the adverse event rate associated with the currently licensed live vaccinia virus vaccine makes reinstatement of policies recommending routine pre-exposure vaccination unlikely in the absence of an orthopoxvirus outbreak. Consequently, licensing of safer vaccines and therapeutics that can be used post-orthopoxvirus exposure is necessary to protect the global population from these threats. Variola virus is a solely human pathogen that does not naturally infect any other known animal species. Therefore, the use of surrogate viruses in animal models of orthopoxvirus infection is important for the development of novel vaccines and therapeutics. Major complications involved with the use of surrogate models include both the absence of a model that accurately mimics all aspects of human smallpox disease and a lack of reproducibility across model species. These complications limit our ability to model post-exposure vaccination with newer vaccines for application to human orthopoxvirus outbreaks. This review seeks to (1) summarize conclusions about the efficacy of post-exposure smallpox vaccination from historic epidemiological reports and modern animal studies; (2) identify data gaps in these studies; and (3) summarize the clinical features of orthopoxvirus-associated infections in various animal models to identify those models that are most useful for post-exposure vaccination studies. The ultimate purpose of this review is to provide observations and comments regarding available model systems and data gaps for use in improving post-exposure medical countermeasures against orthopoxviruses.

Keywords

Smallpox vaccination; Prophylactic vaccination; Animal models; Post-exposure vaccination; Orthopoxviruses; Variola virus; Epidemiology; Smallpox; Monkeypox; Vaccinia

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1. Introduction

1.1. Overview

This review has three major goals: (1) to summarize conclusions about the efficacy of post-exposure smallpox vaccination against clinical disease presentation from historic epidemiological reports and modern animal studies; (2) to identify data gaps in these studies; and (3) to summarize the clinical features of orthopoxvirus-associated infections in various animal models in order to identify those models that are most useful for post-exposure vaccination studies.

1.2. The origins of modern smallpox vaccines

Smallpox vaccination using a heterologous species of orthopoxvirus (OPXV) became common practice after Edward Jenner's famous experiment in which he inoculated a young James Phipps with material from a cowpox (CPXV) lesion in 1796 [1]. Early reports on the effectiveness of pre-exposure vaccination were confirmed by well-documented studies performed throughout the 19th century which demonstrated significantly lower rates of smallpox mortality in geographic areas with mandatory vaccination as opposed to areas where vaccination was not required [2].

1.3. The role of vaccination in the eradication of smallpox

One major contribution to the eradication of smallpox was the availability of an effective live vaccinia virus (VACV) vaccine. Vaccination was utilized pre-exposure to prevent smallpox infection and post-exposure during smallpox outbreaks to vaccinate potentially exposed contacts of infected patients. This methodology coupled with the strict isolation of patients was successful in protecting those contacts from severe disease and producing a "ring" of protection that halted disease transmission. Other factors, including the lack of a reservoir for variola virus (VARV), the development of a heat-stable vaccine, the introduction of the bifurcated needle, and a disease course which allowed time for post-exposure vaccination to elicit a protective immune response, all contributed to the development and implementation of the smallpox eradication effort [3]. Because of widespread pre-exposure vaccination, serious adverse events (SAEs) of smallpox vaccination were well known by the early 20th century but the more significant threat of endemic smallpox ensured that mass vaccination campaigns remained an important defense against outbreaks [4,5]. As eradication efforts progressed, it became apparent that eradication goals could not be met until surveillance systems, systematic investigation of outbreaks, and post-exposure isolation and vaccination were all successfully implemented [6,7]. As cases of smallpox declined, the relative risk of SAEs associated with 1st generation vaccines (vaccines utilizing live VACV propagated on livestock) rose, which led to the recommendation that mandatory vaccination be halted. This was done in the United States in 1971 and worldwide by 1980, when smallpox was officially declared eradicated by the World Health Organization (WHO) [8]. The efficacy of pre-exposure vaccination using these 1st generation vaccines in preventing smallpox disease was well documented during the eradication era. However, post-exposure vaccination with 1st generation vaccines, while generally believed to be at least partially protective, remains less defined, which makes the evaluation of the efficacy of newer and future vaccines more complicated.

1.4. Post-exposure vaccination as a medical countermeasure

The cessation of mandatory prophylactic vaccination has resulted in over half of the global population being potentially naïve to OPXV threats. Despite decades of continuous research to increase vaccine safety without a loss in efficacy, and the creation of 2nd generation vaccines (live VACV propagated in cell lines), 3rd generation vaccines (attenuated VACV) [9] and subunit vaccines [10,11]; only one vaccine (Acambis 2000) has been licensed for use at this time [12]. However, the use of Acambis 2000 continues to be limited due to its adverse event profile [13]. Recommendations to vaccinate U.S. health care workers and laboratorians have previously met with low compliance rates, largely due to the known risk of SAE's following vaccination [14]. In addition, a sizable proportion of the global population is contraindicated for vaccination with Acambis 2000 due to various health conditions [15]. The development of medical countermeasures and safer vaccines that are efficacious against OPXV is an ongoing effort – one which requires an understanding of 1st, 2nd, 3rd and subunit vaccine efficacy in both pre-and post-exposure scenarios [11].

1.5. Assessment of the threat of OPXV-related diseases

Medical countermeasures to OPXVs are important because smallpox re-emergence through a release of VARV would be a high-consequence event (although the risk of this happening is perceived to be low), and because emerging and re-emerging zoonotic OPXV-associated diseases continue to be a public health issue. The WHO Commission to Certify Smallpox Eradication instituted an international surveillance program for smallpox-like diseases in 1971 [16], which ultimately resulted in an increased awareness of human monkeypox virus (MPXV) infection [17]. Today, MPXV infections are on the rise in the Democratic Republic of the Congo (DRC) [18], and outbreaks in Sudan and the United States indicate the potential for MPXV to spread [19]. Other OPXV infection outbreaks are routinely observed and include VACV in Brazil [20], CPXV in Europe [21], and buffalopox in India [22]. Current research also indicates that OPXV in wildlife reservoirs is more prevalent than previously thought [23–25]. Lastly, long-held concerns regarding the threat of smallpox as a weapon of bioterrorism increased after the events of September 11, 2001 and the subsequent anthrax releases [26]. Combined, these conditions make the development of medical countermeasures against OPXV-associated disease an ongoing and current research effort.

1.6. Future vaccine research

During the global eradication of smallpox, a wealth of epidemiologic data was collected. These data have subsequently informed public health practices regarding response strategies to outbreaks of OPXV-associated diseases. However, in the absence of smallpox disease, evaluating the efficacy of newer medical countermeasures – to include 2nd and 3rd generation as well as subunit vaccines – will likely depend on information derived from laboratory, rather than clinical, research [16,27]. The development and utilization of surrogate models will play an important role in the testing of newer vaccines for efficacy in both prophylactic and post-exposure/post-challenge scenarios against systemic OPXV infections [28,29].

1.7. Summary

The data presented in this review can be used to identify those surrogate models that are most useful for post-exposure vaccination studies, inform policy and practice, identify additional research needs and offer insight into the use of post-exposure vaccination to control zoonotic OPXV outbreaks and potential VARV release.

2. Historical epidemiological reports

2.1. Efficacy of post-exposure vaccination

Historical reports on outbreaks of variola major offer insight to the use of post-exposure vaccination as a medical countermeasure to OPXV exposure. As early as 1904, it was well known that vaccination of persons exposed to smallpox helped protect these persons from smallpox disease [30], but the literature is rife with conflicting information about the timing of effective post-exposure vaccination. A summary review of the epidemiological literature which specifically mentions post-exposure vaccination efficacy in the context of variola major outbreaks [31–42] highlights historic estimations of the efficacy of vaccination at various timepoints post-exposure (Table 1). This literature is complicated to interpret due to various data gaps, which have also been detailed in Table 1.

Historical data in the early 20th century indicated to some researchers that post-exposure vaccination is not effective in preventing disease in immunologically naïve persons when administered at any timepoint post-exposure [43], while other researchers argued that primary vaccination administered post-exposure was completely protective if administered <1 day post-exposure, partially protective at up to 3 days post-exposure, but not protective after 3 days post-exposure [44]. This latter view is supported by more recent retrospective analyses. A DELPHI analysis of expert opinion [45] suggested post-exposure vaccination within 24 h would be 90% effective, and within 1–3 days post-exposure would be 80% effective, in disease prevention. In those who did develop disease, disease severity was also estimated to be minimized. Vaccination up to a week post-exposure was also estimated to have partial benefit. A review of four British clinical summaries of post-exposure vaccinations provided from the late 1800s through the early 1900s which did not prevent smallpox disease do indicate that post-exposure vaccination within 3 days post-exposure is effective at decreasing disease severity in those not previously vaccinated [46]. Fig. 1 is a generalized summary of data from the epidemiological reports reviewed in Table 1 and is designed to visually chart the longest period post-exposure where vaccination which was reported to increase survival and/or to reduce disease severity in each report. As can be seen in Fig. 1, these data suggest that the administration of post-exposure vaccination to contacts decreases mortality and/or reduces morbidity in 100% (12/12), 83% (10/12), 75% (9/12), 67% (8/12), 58% (7/12), 42% (5/12), 33% (4/12) and 25% (3/12) of reports when vaccine is given at <1, <2, <3, <5, <7, <9, <10 and <12 days post-exposure, respectively. Due to the data gaps described below, the data summary in Fig. 1 is a qualitative representation of these reports.

2.2. Identification of data gaps

In general, the epidemiological data from variola major outbreaks before and during the eradication era appear to support the current view that vaccination prior to 3 days post-exposure will provide benefit in preventing smallpox disease in exposed persons regardless of prior vaccination status. In addition, it appears that post-exposure vaccination given prior to the appearance of rash affords clinical or survival benefit, but these benefits diminish when vaccine is administered greater than one-week post-exposure. However, it is important to note that these data are limited in many ways.

During this period only 1st generation vaccines were used; prior immunity in individuals within the population (whether from previous vaccination or exposure) may be underestimated; underlying immune issues of patients are unknown; specific descriptions (i.e. Rao's classification) of the clinical presentation of index patients and contacts that develop disease are not included; vaccine quality and administration differences may be present as vaccine quality prior to 1971 was largely uncontrolled and the bifurcated needle was not recommended until 1968 [47,48]. Additionally, patient demographics as well as social/cultural/economic differences are also not always identified. Any one of these missing pieces of information could help explain the variability among – and affect interpretation of – these studies. Consequentially, our conclusions are tempered with the understanding that these patients are presumed to be naïve when given post-exposure vaccination, are presumed to be immunocompetent, are presumed to be exposed to sufficient VARV to cause infection and disease, and are presumed to have been given a quality vaccine using appropriate administration routes. These presumptions and data gaps make it difficult to interpret the true efficacy of post-exposure vaccination. In the absence of human smallpox disease, surrogate models offer our best chance of defining when post-exposure vaccination will provide a survival benefit. The next section of this review will examine the limited literature regarding post-exposure vaccination studies in surrogate models.

3. Current laboratory studies in surrogate models

3.1. Efficacy of post-exposure vaccination

Although both pre-exposure vaccination and post-exposure antiviral therapy studies in surrogate models contribute to our understanding of outcomes following OPXV challenge, for the purposes of this review, only those surrogate model studies that have provided information regarding the efficacy of post-exposure vaccination are addressed. The data from these few reports are highly variable depending on the surrogate model used. In contrast to the human epidemiologic literature, where “post-exposure” may not necessarily mean post-infection, in the animal models, all post-exposure data on vaccine efficacy is “post-infection”. Studies that *did not* show a significant survival benefit for post-exposure vaccination include a study where 2.5×10^5 TCID₅₀ of VACV-Elstree (intracutaneous) was administered 1 day post-exposure in cynomolgous macaques infected with 10^7 pfu of MPXV (intratracheal) without any significant survival benefit when compared to control animals [49]. In a second study, 10^6 pfu of Elstree (scarification) or 10^8 IU of Modified Vaccinia Ankara (MVA) (intramuscular) were administered post-exposure to BALB/C mice which were challenged with 10^4 or 10^6 pfu (VACV-Western Reserve) (respiratory)

respectively. The authors report that although MVA administration within 3 h of challenge protected the mice from death, these animals still manifest substantial disease symptoms. There was no significant survival benefit with MVA vaccination on day 1, 2, 3 or 4 and none with VACV-Elstree vaccination on day 0, 1 or 2 post-challenge [50].

Studies that *did* show a survival benefit for post-exposure vaccination used a murine model with an intranasal (respiratory) challenge using ectromelia virus (ECTV). This model has a longer disease course (mean time to death 10 days vs. 6 days) and longer incubation period (time to initial weight loss 7 days vs. 2–3 days) than VACV-WR intranasal (respiratory) infection of BALB/C or C57BL/6 mice. Using C57BL/6 mice lethally challenged with ECTV at $5 \times \text{LD}_{50}$ (respiratory), 100% survival was observed after 10^6 pfu VACV-Lister (intramuscular) vaccination at 0 and 1 day post-exposure and a smaller survival benefit of 40% was observed with vaccination 2 or 3 days post-exposure. Intramuscular administration of 1×10^8 pfu of MVA on day 0, 1 or 2 post-exposure demonstrated 100% protection while day 3 vaccination resulted in 80% survival benefit. In this same study, the use of BALB/c mice in a $3 \times \text{LD}_{50}$ challenge with ECTV (respiratory), coupled with vaccination with 10^6 pfu VACV-Lister (intradermal tail scarification) resulted in 100%, 83% and 16% survival when administered on day 0, 1 or 2 post-exposure. In a second experiment, 10^6 pfu VACV-Lister (intramuscular) given on days 0, 1, 2, 3, or 4 post-exposure, survival benefit was 100%, 80%, 40%, 20% and 20%. MVA proved more effective with 10^8 pfu of MVA (intramuscular) resulting in survival benefits of 100%, 100%, 100%, 60% and 20% when given on day 0, 1, 2, 3 or 4 post challenge. When C57BL/6 mice were used in the $3 \times \text{LD}_{50}$ ECTV challenge, all animals (100%) survived when 1×10^8 pfu MVA vaccination (intranasal) was given on day 0 or day 1 with day 2 post-exposure vaccination providing 50% survival [51]. In a second study, C57BL/6 mice were infected with a lethal dose of 3×10^4 TCID₅₀ ECTV (intranasal) and then vaccinated on day 2, 3, or 5 with 5×10^7 TCID₅₀ of MVA (intranasal). The survival benefit was 30% for day 2 vaccination and 0% for day 3 and 5 vaccination. However, 100% survival was seen with 5×10^7 TCID₅₀ MVA (intravenous) vaccination on day 2 and day 3 and 20% survival was seen with vaccination on day 5 [52]. In the final study reviewed here, mice with a deficient innate immune response (TLR9^{-/-}) were lethally challenged with 100 TCID₅₀ ECTV (respiratory) and received 1×10^8 TCID₅₀ MVA (intranasal) 24 h and 48 h post-exposure with 100% survival benefit. The same study using 72 h post-exposure vaccination showed a 30% survival benefit [53]. Fig. 2 graphs the combined survival benefits shown in each of the aforementioned experiments by day of post-exposure vaccination.

In summary, these results indicate no protective effect of post-exposure vaccination with intradermal administration of 1st generation, or subcutaneous administration of 3rd generation, vaccines for either the MPXV (intratracheal) macaque model or the VACV-WR (respiratory) challenged mouse model – both of which have rapid onset of symptomatic illness. In contrast, protection by post-exposure vaccination was seen in the ECTV (respiratory) challenged BALB/C or C57BL/6 mouse models. Protection was beneficial for a longer period post-exposure if 1st generation smallpox vaccines were administered intramuscularly rather than intradermally (tail scarification), or when 3rd generation vaccines were given via intranasal, intramuscular or intravenous routes of administration.

The studies above also provide data regarding immune responses related to short-term pre-exposure and post-exposure vaccination in the ECTV model. One study utilized Rag-1^{-/-} and IFNAR^{-/-} mice vaccinated with 1×10^8 pfu MVA (intranasal) 2 days prior to ECTV challenge to demonstrate that Type IIFN is important but not essential for MVA-induced protection, whereas adaptive immunity is essential. In animals given either 1×10^6 or 1×10^8 pfu of VACV-Lister or MVA (intramuscular), a higher dose of either vaccine resulted in more antibodies being made earlier. This study also demonstrated that the titer of antibodies necessary for passive protection (3×10^4) is higher than the titer induced by vaccination in this model [51]. In an additional study, ECTV was shown to depend on TLR9 recognition in order to stimulate innate immunity, whereas MVA could stimulate innate immunity without TLR9 [53]. C57BL/6 mice challenged with a $14 \times$ LD50 dose of ECTV and vaccinated with MVA (intranasal and intravenous) on day 3 post-exposure were only protected with intravenous vaccination. In these protected animals activation of innate and cell mediated immunity shown by increased cytokine production and activation of NK and T cells was higher than in non-protected animals. In animals with intravenous vaccination IgG neutralizing titers could be detected earlier (day 6) and had higher titers ($\sim 10^3$) than in intranasally vaccinated animals (day 9) and ($\sim 10^2$). The similarity of results between unvaccinated and intranasally vaccinated animals indicates that the immune response (both innate and adaptive) to the original ECTV challenge was not enhanced by intranasal vaccination [52].

3.2. Identification of data gaps

As the results above demonstrate, multiple variables contribute to the difficulty in interpreting surrogate model studies. These variables include differences in disease course and immune responses - which are, in turn, induced by different challenge viruses, vaccines, routes of infection, routes of vaccine administration, doses of virus and doses of vaccine. In the studies reviewed above, there is variability among models and even within the ECTV infected mouse model – results are consistent for day 0 and day 1 vaccination but not day 2, 3, 4 and 5 vaccination. This may be due to differences in BALB/C and C57BL/6 mice or may reflect differences in vaccine routes of administration. The differences between VACV or ECTV disease in mice and human systemic OPXV disease do not permit a full evaluation of the potential effect of post-exposure vaccination on human disease. Lastly, intranasal or intravenous administration of post-exposure vaccination has not been studied in humans making the applicability of these studies difficult to ascertain. Therefore, while these studies have been the first to address the issue of post-exposure vaccination and certainly inform the field on this issue, additional animal models – preferably those models with an extended incubation period – and diseases courses, which better mimic human disease – will enable better predictions per the human experience. The next section of this review covers those surrogate models that are currently in use that would be most suitable for future post-exposure vaccination studies.

4. Comparative analysis of human and surrogate model disease courses

4.1. Identification of surrogate models for post-exposure studies

Because of the data gaps identified above in both historical epidemiology reports and current surrogate model data, more laboratory studies in different surrogate models may help to define the window of time in which post-exposure vaccination offers a survival benefit. Because there is no non-human reservoir for VARV [54], it is not possible to use a naturally infected species as a surrogate for human systemic OPXV infection [55]. Fortunately, scientists have been developing, testing, evaluating and improving surrogate models that use non-VARV OPXVs to challenge numerous animal species since the beginning of the 20th century [56,57]. While much progress has been made, many models are difficult to compare to human disease and most do not fulfill all the requirements of the Federal Drug Administration's (FDA) animal rule, which provides the current standards that surrogate models must meet in order to replace human efficacy trials for FDA licensure [58]. The major requirements of this regulation are that there is (1) a reasonably well understood mechanism for the pathogenesis of the challenge virus, (2) an understanding of the correlates of protection for vaccination and (3) that these are demonstrated in more than one animal species. While many surrogate models share similarities with systemic OPXV disease in humans, none of the individual models fully recapitulates smallpox disease in humans. The majority of models have very short incubation times and rapid disease courses, which make them less desirable for post-exposure vaccination studies, therefore we have limited our review to those models that offer an incubation period of at least 5 days and in which previous studies have indicated vaccine efficacy of some kind [59–79].

The purpose of our analysis is to identify those models that appear to be most relevant for extrapolation to humans for the development of public health policy and research in the absence of pre-exposure vaccination and human VARV disease. Within the subgroup of currently used surrogate models that have an extended incubation period and previous vaccine efficacy data, we have given priority to those models that (1) utilize an etiological agent that is a human pathogen – in order to better compare mechanisms of pathogenesis between the model and humans, (2) exhibit rash illness and ~30% mortality – as rash illness is an important marker of morbidity and that coupled with realistic mortality allows for the fine analysis of the immune components of partial protection and (3) can be vaccinated with current vaccines at similar doses and routes of administration which have been tested in human trials - again, in order to clarify the comparisons between models and humans. Table 2 details the clinical features of the incubation, prodrome, rash and resolution stages of OPXV infections in selected models. A comparative analysis of the disease course of smallpox in humans to the disease course of OPXV infections in these surrogate models allows for the identification of models that can be utilized most effectively for post-exposure vaccination efficacy studies.

4.2. Comparative analysis of surrogate models for post-exposure vaccination studies

Based on epidemiological data from the smallpox era, post-exposure vaccination is likely to be most efficacious when administered during the incubation period. As stated above, we have chosen only to consider those models demonstrating incubation periods of more than 5

days that have also previously been utilized to evaluate either pre- or post-exposure vaccination. This selection criteria led to the exclusion of the Rabbitpox – New Zealand White Rabbits (aerosol) or Variola – cynomologous macaque (intravenous) models due to short incubation periods [80–82]. The recently developed Calpox – marmoset (intranasal) [83,84] model shows promise for post-exposure vaccination modeling but was excluded from this review because no vaccine testing has occurred in this model at this time.

Each of the models in this table has weaknesses that limit their effectiveness, suggesting that post-exposure vaccination studies should be conducted in multiple surrogate models. The rhesus and cynomologous macaque model of VARV infection (aerosol) is a model with very low mortality, thus making it difficult to show survival benefit of post-exposure vaccination. The cynomologous macaque model of MPXV infection (aerosol) has differences in disease presentation when compared to human infections and has an abbreviated incubation period and high mortality (100%) when compared to the cynomologous macaque model of MPXV infection (intratracheal microspray aerosol) which has 33% mortality and a more similar disease course to humans with systemic OPXV infections. While the non-human primate model is the most studied of the smallpox surrogate models because these animals have a close evolutionary relationship to humans and reagents are readily available, there are several practical constraints to widespread use. For example, the expense, training and facility requirements associated with non-human primate studies limits the number of institutions that can perform research using these models and can occasionally result in underpowered experiments. While this concern can be partially offset by the use of the aforementioned highly lethal non-human primate models, these typically have a rapid disease onset and do not allow for an understanding of the kinetics of the immune response generated by post-exposure vaccination. In addition, regulations stipulate that potential therapies be proven efficacious in multiple animal models, which suggests the need for small animal models to complement non-human primate models.

Immunological reagents are also commercially available for mice and the availability and relatively low cost of mouse studies make them an attractive small animal alternative. The ectromelia virus (ECTV) (intranasal) infected mouse model has high lethality and dissimilar disease presentation when compared with human infections and ECTV is not virulent in humans. A better mouse model appears to be the MPXV infected (intranasal) CAST/EiJ model that has an extended incubation period when compared to the ECTV model. Unfortunately, this model also has a disease presentation that differs from human systemic OPXV infections in that animals do not demonstrate rash illness, which is an important marker for determining the effect of post-exposure vaccination on morbidity. Proportional mortality and presence of rash illness are especially important when attempting a fine analysis of the immune components of partial protection, vital for evaluation of smallpox vaccines in immunocompromised humans.

Lastly, the outbreak of monkeypox in the United States in 2003 offered a unique opportunity to evaluate exotic surrogate models, which are relatively inexpensive, outbred, natural hosts of MPXV. However, the lack of commercially available immunological reagents is a major weakness of these models. The MPXV infected (intranasal) prairie dog model has the advantage of an extended incubation period, similar disease presentation to infected humans

to include rash illness and 30–50% mortality. This is superior to the ground squirrel model, which has high lethality and atypical disease presentation. Therefore, although each of the models in Table 2 are suitable for post-exposure vaccination studies, the MPXV infected (intratracheal microspray aerosol) cynomolgous macaque, the MPXV infected (intranasal) CAST/EiJ mouse and the MPXV infected (intranasal) prairie dog offer distinct advantages in terms of disease presentation, challenge virus used and extended incubation periods for post-exposure vaccination testing.

5. Conclusions

Examination of the studies reviewed here demonstrates multiple issues in the evaluation of post-exposure vaccination efficacy against OPXV threats. Data gaps identified in the historical epidemiological reports (Table 1 and Fig. 1) make it difficult to determine a post-exposure efficacy window for human infection and do not reflect changes in immune status of the current human population. The data suggest that vaccination prior to 3 days post-exposure will have significant benefit in minimizing smallpox disease in exposed persons and post-exposure vaccination any time prior to the appearance of rash may afford a slight survival benefit. However, it is difficult to compare this data to the studies performed in surrogate models (Fig. 2) for several reasons.

In animals with a somewhat accelerated disease course such as ECTV challenged mice (mean time from challenge to death = 10 days, time to weight loss 7 days), post-exposure vaccination with 1st generation vaccines via intradermal tail scratch at 1 or 2 days post-exposure/infection is 83% and 16% protective, respectively. In human smallpox infections (mean time from exposure to death = 20 days (16–23 days)) [61], post-exposure vaccination at equivalent time points of <2 or <4 days are beneficial in 80% and 60% of reports. This simple comparison highlights the difficulties of interpreting data in models with an accelerated disease course and comparing those results to human smallpox data. Similarly, rash illness is an important marker of morbidity in the most common types of human smallpox cases, where mortality is ~30%. Models that lack rash illness, or have very high or very low mortality, produce data that are more difficult to extrapolate to post-exposure vaccination efficacy in humans.

Post-exposure vaccination is only efficacious if the vaccine elicits a protective immune response against OPXV infection prior to the point at which disease prevention or modification is no longer preventable. Only one study has directly compared differences between failed post-exposure vaccination protection (MVA-intranasal) and successful post-exposure vaccination protection (MVA-intravenous) in the ECTV model. Results of that study indicate that activation of innate and adaptive immunity shown by increased cytokine and neutralizing antibody production as well as activation of NK and T cells is important for post-exposure vaccination protection [52]. However, a more recent study in the ECTV model indicates that B cells, but not T cells, are dispensable in protecting animals vaccinated with MVA (intranasal) and then challenged 2 days later with a $3 \times LD_{50}$ dose of ECTV [85]. While these data are consistent with innate and cell mediated immunity playing a large role in protection from primary infection they also illustrate the importance of understanding the

differences in protective immune responses that are induced by pre-exposure (long-term and short-term) vaccination and post-exposure vaccination.

Neutralizing antibody production in vaccinated humans has been shown to correlate with expression of various genes associated with innate and cell-mediated immunity, indicating the integral nature of the adaptive immune system in humans [86]. Mice that are incapable of producing poxvirus specific IgG and IgM are not protected by post-exposure MVA vaccination (intravenous) at day 3 post-exposure whereas wild type mice are which indicates the importance of neutralizing antibodies [52]. The time it takes a virus to spread to the point where post-exposure vaccination is not beneficial will depend in part on the incubation period of the virus and the kinetics of the immune response. For example, primary exposure requires 7–13 days to produce neutralizing antibodies against vaccinia in human studies [87–89], and smallpox in humans has a combined incubation and prodromal period that lasts 7–17 days with a mean of 12.5 days [90]. Based on these data, smallpox contacts who have been exposed and have a shorter incubation period (<12.5 days) may not have time to mount a neutralizing antibody response to the VARV exposure and thus supplementing this response with a post-exposure vaccination may gain no protective benefit. Non-human primate studies demonstrate antibody formation after primary vaccination that is slightly faster than humans, with titers rising by day 6 and peaking by day 9–10 [91,92]. In mice, VACV infection induces neutralizing antibodies as early as 4 days post-challenge, while ECTV induced neutralizing antibodies appear by day 6 and peak around day 9 [52,93]. Anti-VACV neutralizing antibodies have been shown to arise between days 7 and 10 post MPXV challenge in the prairie dog [77]. This illustrates the importance of an adequate incubation period to understanding the kinetics, magnitude and breadth of the antibody response after post-exposure vaccination and its correlation to protection.

An additional difficulty in bridging the data gaps between human and surrogate models involves the route of administration of challenge virus and vaccine. The models selected here (MPXV-macaque (intratracheal microspray aerosol), MPXV-CAST/EiJ mouse (intranasal) and MPXV-prairie dog (intranasal) all use a challenge virus that causes a systemic infection and can be vaccinated using the same route of administration that is used for human vaccination. Reports indicate that the route of administration of challenge virus and vaccine affects the magnitude of neutralizing antibodies [94]. In addition, protection from systemic or localized disease requires different antibody targets [95] making this an important aspect of surrogate model design. As human studies of vaccination must rely on challenges that utilize non-systemic infections (i.e. challenge with vaccines), surrogate models that exhibit systemic infection are important.

The data gaps identified in this review for both epidemiological reports from the eradication era and more modern surrogate studies indicate a need for additional studies in surrogate models. Although it has been challenging to implement the FDA animal rule for product review [96], the regulatory and scientific communities have made significant progress in clarifying what is needed to provide the appropriate data for use in regulatory product review [12]. The three models identified previously in this review as most advantageous for post-exposure vaccination studies (MPXV-macaque (intratracheal microspray aerosol), MPXV-CAST/EiJ mouse (intranasal), and MPXV-prairie dog (intranasal)) meet many of the

requirements for surrogate models. Each of these use a human pathogen as a challenge virus, routes/doses of vaccine that are similar to those tested in human vaccination trials and routes of challenge that approximate respiratory infection in humans. Lastly, the macaque and prairie dog models offer a disease presentation that most closely mimics human infection, the prairie dog and mouse models are relatively cost-effective and the mouse and macaque models have the best availability of immunological reagents for determining the correlates of protection in post-exposure vaccination studies. Therefore, a combination of post-exposure studies using all three of these models offers our best chance of not only understanding post-exposure vaccination efficacy but also successfully testing new vaccines against OPXV infection in a post-exposure setting.

The question of the efficacy of post-exposure vaccination against smallpox is not a trivial one. “Sound administrative procedure must depend on accurate knowledge of epidemiology; once the latter is defined the former becomes clear” [31]. While the historical human data from the eradication era is and has been indisputably important, it represents smallpox vaccination with 1st generation vaccines in a global population where immunosuppression by HIV infection and medical treatments were relatively low. Without modern epidemiologic information on human cases of smallpox in today’s population using newer vaccines, policymakers are designing medical countermeasures against OPXVs in the absence of human infection data. To make informed decisions, research must continue to bridge the gaps between historic smallpox epidemiology and laboratory research. This can only be accomplished through the wise use of current surrogate models and the development of improved models.

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Table 1 References - Year of Outbreak

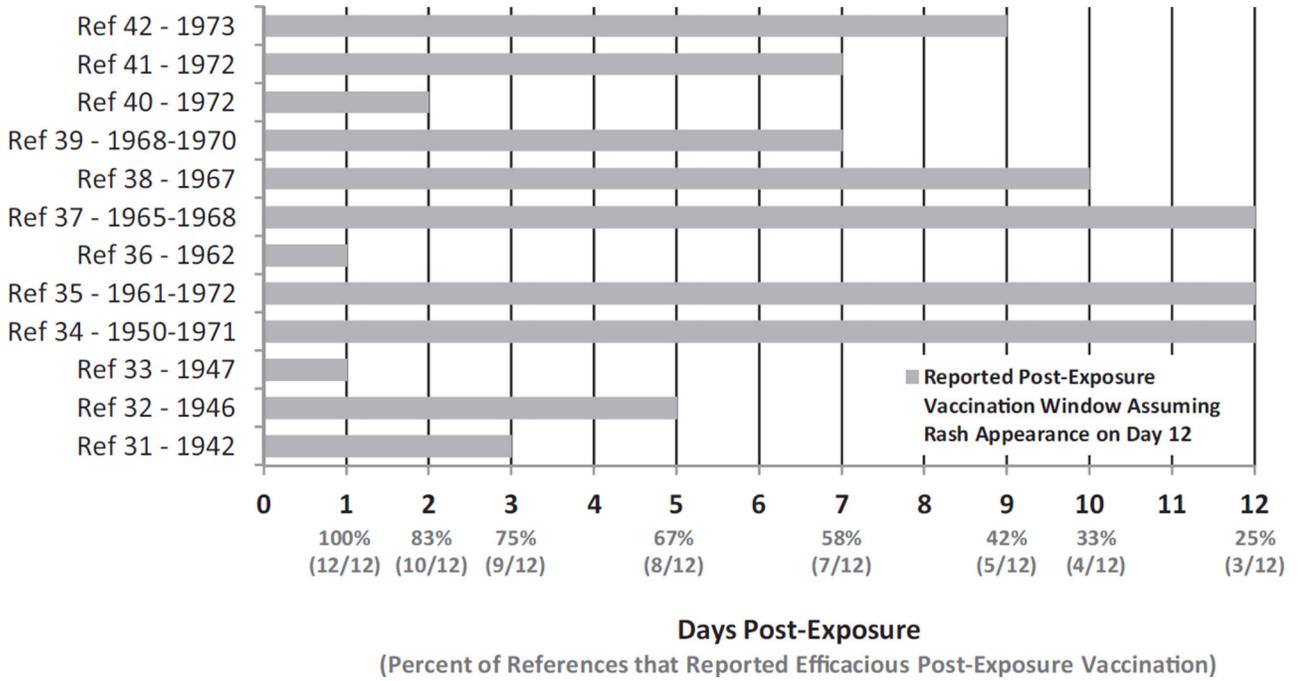
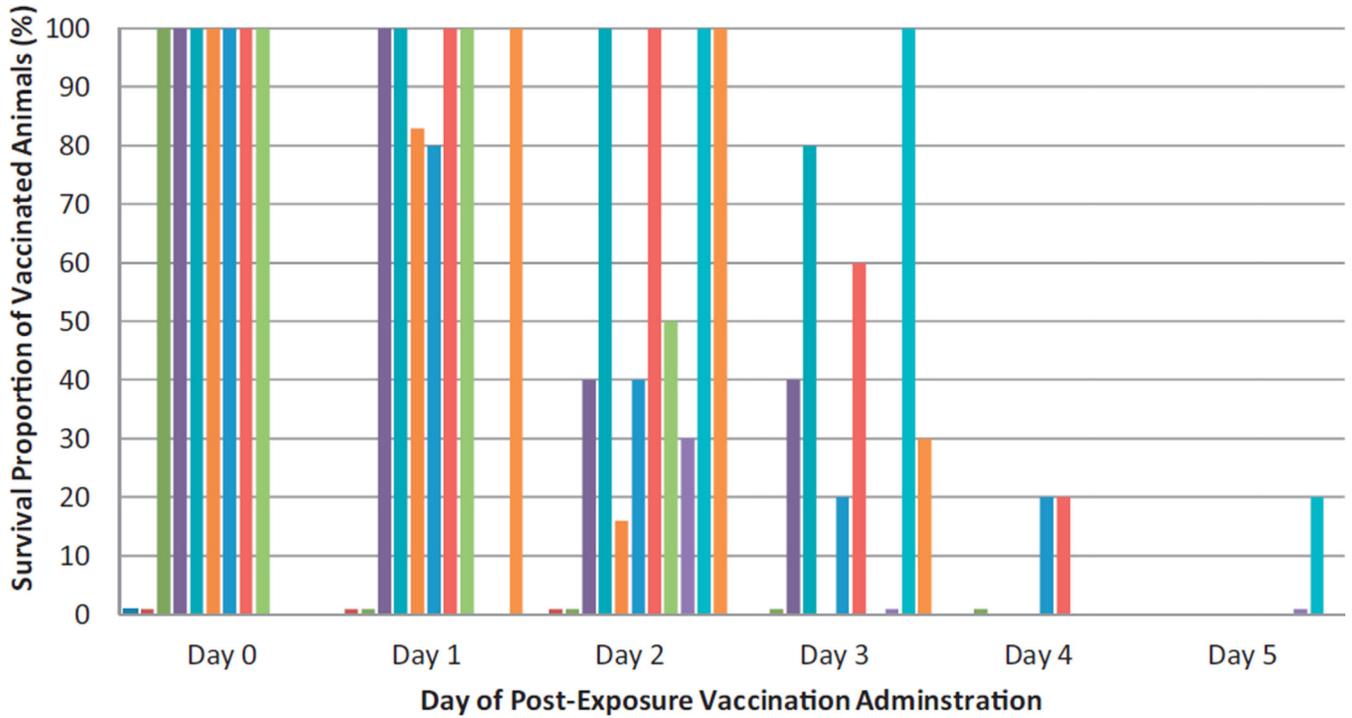


Fig. 1.

Post-exposure vaccination window extrapolated from historical epidemiology reports. A review of references that described post-exposure vaccination as providing partial or complete protection from, or attenuation of, smallpox symptoms during disease outbreaks was accomplished. The reported post-exposure vaccination windows were charted (gray bars) by the days post-exposure that efficacious vaccination was administered (black horizontal axis text). The percentage of references that indicated a benefit to post-exposure vaccination prior to each day post-exposure (gray horizontal axis text) were determined.



- Ref 49 - MPXV-Macaques (intratracheal) : VACV-Elstree (intracutaneous)
- Ref 50 - VACV-WR-BALB/C Mice (respiratory) : VACV-Elstree (scarification)
- Ref 50 - VACV-WR-BALB/C Mice (respiratory) : MVA (intramuscular)
- Ref 51 - ECTV-C57BL/6 Mice (respiratory) : VACV-Lister (intramuscular)
- Ref 51 - ECTV-C57BL/6 Mice (respiratory) : MVA (intramuscular)
- Ref 51 - ECTV-BALB/C Mice (respiratory) : VACV-Lister (intradermal tail scarification)
- Ref 51 - ECTV-BALB/C Mice (respiratory) : VACV-Lister (intramuscular)
- Ref 51 - ECTV-BALB/C Mice (respiratory) : MVA (intramuscular)
- Ref 51 - ECTV-C57BL/6 Mice (respiratory) : MVA (intranasal)
- Ref 52 - ECTV-C57BL/6 Mice (intranasal) : MVA (intranasal)
- Ref 52 - ECTV-C57BL/6 Mice (intranasal) : MVA (intravenous)
- Ref 53 - ECTV-TLR9-/- Mice (respiratory) : MVA (intranasal)

Fig. 2. Post-exposure vaccination window extrapolated from surrogate model studies. References that reported post-exposure vaccination studies were reviewed. The proportion of animals that survived lethal challenge (vertical axis) is given for each surrogate model tested (gray bars) by the day post-exposure vaccination (horizontal axis) was administered for each individual experiment.

Table 1

Summary of literature review of post-exposure vaccination efficacy against smallpox.

Year(s) of variola major outbreak	Location(s) of variola major outbreak	Outbreak case fatality rate (all cases)	Original author conclusions regarding the efficacy of post-exposure vaccination in previously unvaccinated contacts	Data gaps affecting interpretation of conclusions ^a	Ref.
1942	Glasgow, Edinburgh and Fife, Scotland	24%	Patients vaccinated day 0–3 post-exposure had significant improvement in fatality and reduction in severity of disease. "Number of cases of smallpox in recently vaccinated contacts was small".	Summary article, "reduction in severity," unexplained, quantitative results not reported.	[31]
1946	Tripolitania, Libya, North Africa	18%	Contacts vaccinated 1–5, 6–10 or 10+ days after contact had mortality rates of 0%, 19% and 25% respectively. Patients vaccinated within 5 days of exposure had milder disease.	Small number of post-exposure vaccinees.	[32]
1947	Bilston, England	20%	Vaccination ~3–4 days post-exposure did not influence disease course. Modified cases of smallpox occurred where vaccination had been carried out 0–1 day post-exposure.	Survival of confluent cases unknown, quantitative results not given.	[33]
1950–1971	Cases imported into Europe	16%	The case fatality rate of patients vaccinated post-exposure was 29% - compared to a fatality rate of 52% in those that were never vaccinated.	No explanation of the timing of post-exposure vaccination is given.	[34]
1961–1972	Madras, India	43%	Vaccination post-exposure in 426 cases of ordinary smallpox resulted in a case fatality rate of 20.6% compared to a case fatality rate of 36.9% in 1296 unvaccinated persons.	Timing of post-exposure vaccination is unknown. Small number of post-exposure vaccinees.	[35]
1962	Bradford, England	50%	Vaccination within 24 h of exposure resulted in mild forms of disease. Primary vaccination on day 11 post-exposure was ineffective.	Descriptive case studies only, small number of relevant cases, outbreak had higher than normal fatality rate.	[36]
1965–1968	Madras, India	Not given	In this study of familial contacts, 47.6% of unvaccinated contacts developed smallpox, while only 29.5% of those contacts that received primary vaccination did. Of those 29.5%, those cases (excluding pregnant women) that were vaccinated after exposure also showed decreases in hemorrhagic disease presentation and increases in modified disease presentation.	No mention of fatality rates for outbreak, no discussion of when post-exposure vaccination was provided (day 1, 2, 3 post exposure etc.). Data acquired during mass vaccination campaign	[37]
1967	Sheikhupura District, Punjab, West Pakistan	Not given	75% (12/16) of those who were vaccinated within 10 days of contact developed smallpox while 96% (26/27) of those not vaccinated within 10 days developed smallpox.	No mention of fatality rates for outbreak, small numbers, no discussion of post-exposure vaccination at specific day (day 1, 2, 3 etc.).	[38]
1968–1970	Six rural districts, Punjab, West Pakistan	21%	Of contacts with no prior vaccination history, 78.5% (73/92) of contacts with no post-exposure vaccination developed disease	Small study, no discussion of vaccine efficacy against morbidity. Post-exposure	[39]

Year(s) of variola major outbreak	Location(s) of variola major outbreak	Outbreak case fatality rate (all cases)	Original author conclusions regarding the efficacy of post-exposure vaccination in previously unvaccinated contacts	Data gaps affecting interpretation of conclusions ^a	Ref.
1972	Bangladesh evacuees in Calcutta, India	50%	compared to none (0/2) of the contacts that received post-exposure vaccination within 7 days of exposure. Primary vaccination 0–1 days post-exposure resulted in fatality rate of 37.1%, primary vaccination 2 days post-exposure had fatality rate of 41.9%, compared to 53.4% in the general unvaccinated population.	vaccination efficacy was reported in terms of subsequent attack rates, which assumes that all household contacts are exposed equally, and with similar timing. No discussion of vaccination efficacy against morbidity.	[40]
1972	Khulna Municipality, Bangladesh	27%	Contacts that received primary vaccination 5–7 days post-exposure had an attack rate of 7.9/1000 vs. 14.4/1000 in unvaccinated persons. Overall, 2.4% (10/414) of unvaccinated household contacts who received post-exposure vaccination developed disease compared to 9.2% (5/54) of unvaccinated household contacts who did not receive post-exposure vaccination.	Post-exposure vaccination efficacy was reported in terms of subsequent attack rates, which assumes that all household contacts are exposed equally, and with similar timing. Small number of cases. Not all post-exposure vaccination timing explained.	[41]
1973	Calcutta, India	32%	In patients whose primary vaccination occurred <9 days post-exposure the fatality rate was 41.1% compared to persons vaccinated 9–12 days post-exposure which had a fatality rate of 50% and unvaccinated patients which had a fatality rate of 53.4%.	No discussion of vaccination efficacy against morbidity.	[42]

^a All studies in this table share the following data gaps: vaccine strain not specified, vaccine administration method not specified, vaccine origin and quality not specified, individual patient numbers and demographics not specified, contacts that were vaccinated post-exposure and did not become infected are not discussed.

Table 2

Disease course comparisons between human smallpox infection and surrogate models.

	Disease stages (day of onset)			
	Incubation	Prodrome	Rash	Resolution
Smallpox human exposure dose and strain varies "Respiratory Droplet" [59–61]	Day 0–9 (range 0–16)	Day 10–12 (range 7–17)	Day 12–15 (range 9–20)	Day 26–28 (range 24–37)
	Asymptomatic	Fever, headache, chills, backache, vomiting	Maculopapular to papular within 1–2 days, vesicular by day 4–5 and pustular by day 7 and scabbed by day 14	Death (~30%) or survival and desquamation
Smallpox macaque 10 ⁸ harper particle size <1µm aerosol [62,63]	Day 0–5	Day 5–7	Day 7–8 (range 6–11)	Day 10–11
	Asymptomatic	Malaise, fever, primary bronchopneumonia	Centrifugal distribution papular to vesicular to pustule (within 5 days)	Death (~1%) or survival and desquamation
Monkeypox macaque 10 ⁴ –10 ⁵ pfu zaire 79 particle size = 1.2 µm aerosol [64]	Day 0–6	Absent	Day 6–8	Day 11
	Asymptomatic		Primary bronchopneumonia, papular to vesicular to scabbed (within 3–4 days), hemorrhagic lesions	Death (100%)
Monkeypox macaque 3.4 × 10 ⁶ pfu zaire 79 particle size = 8 µm intratracheal aerosol [65]	Day 0–3	Day 3–8	Day 8–10	Day 12–18
	Asymptomatic	Fever, malaise, lymphadenopathy, weight loss	Primary bronchopneumonia papular to vesicular to pustular to scabbed (within 4–6 days), hemorrhagic lesions	Death (~30%) or survival and desquamation
Ectromelia mice(C57BL/6) 800 pfu Moscow intranasal [66–69]	Day 0–5	Absent	Day 5–7	Day 9–11
	Asymptomatic			Death (~80%)
Monkeypox Mice(CAST/EiJ) 10 ³ pfu MPXV-Z79-CB2 intranasal [70,71]	Day 0–6	Absent	Day 6–8	Day 8–10
	Asymptomatic		No rash, weight loss, ruffling, hunching, lethargy	Death (~60%) or survival
Monkeypox ground squirrel 500–5000 pfu MPXV-Z79-CB2 intranasal [70,72,73]	Day 0–6	Absent	Day 6	Day 6–9
	Asymptomatic		Lethargy, anorexia, frequent nosebleeds, and terminal respiratory distress	Death (100%)
Monkeypox prairie dog 10 ³ –10 ⁴ pfu MPXV-ROC-268 intranasal [74–79]	Day 0–5	Day 5–7	Day 9–11	Day 12–17

Disease stages (day of onset)			
Incubation	Prodrome	Rash	Resolution
Asymptomatic	Weight loss, inappetence	Maculopapular to papular within 1–2 days, vesicular by day 3–4 and pustular by day 4–7 and scabbed by day 9–11	Death (~30–50%) or survival and desquamation

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