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Review

Rabbitpox: a model of airborne transmission of smallpox

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Smallpox is a human disease caused by infection with variola virus, a member of the genus *Orthopoxvirus*. Although smallpox has been eradicated, concern that it might be reintroduced through bioterrorism has therefore led to intensive efforts to develop new vaccines and antiviral drugs against this disease. Because these vaccines and therapeutics cannot be tested in human trials, it is necessary to test such medical countermeasures in different animal models. Although several orthopoxviruses cause disease in laboratory animals, only rabbitpox virus (RPXV) infection of rabbits shows patterns of natural airborne transmission similar to smallpox. Studies have shown that a smallpox-like disease can be produced in rabbits in a controlled fashion through exposure to a small-particle RPXV aerosol, and rabbitpox spreads from animal to animal by the airborne route in a laboratory setting. This model can therefore be utilized to test drugs and vaccines against variola virus and other aerosolized orthopoxviruses.

Introduction

Smallpox is a disease of humans that was eradicated by an intensive worldwide control programme more than 30 years ago (Fenner *et al.*, 1988b). The causative agent of smallpox, variola virus (VARV), is an orthopoxvirus in the family *Poxviridae* and it was transmitted from person to person primarily through inhalation of virus-contaminated respiratory secretions. Concern that smallpox might be reintroduced through bioterrorism has led to renewed efforts to develop medical countermeasures for this disease (Henderson, 1999). Since implementation of the 'Animal Efficacy Rule' by the US Food and Drug Administration in 2002 [regulation 21 Code of Federal Regulations (CFR) 314 Subpart I] for testing vaccines and therapeutics that cannot be studied in human trials, it has become necessary to use different animal models of orthopoxvirus infection as surrogates of smallpox.

Several animal models currently in use rely on injection of virus by the intraperitonal, subcutaneous or intravenous route, or have required large doses of the virus to initiate infection by the airborne or intranasal route (Smee, 2008). Models based on the intravenous challenge of macaques with monkeypox virus (MPXV) or VARV require very high doses of virus (Huggins *et al.*, 2009; Jordan *et al.*, 2009). Mouse models include infection with vaccinia virus (VACV), ectromelia virus or cowpox virus by an intranasal or aerosol route (Smee *et al.*, 2001; Bray *et al.*, 2002; Schriewer *et al.*, 2004). Recent models also include MPXV in STAT-1 knockout mice, dormice, prairie dogs or ground squirrels by intranasal, subcutaneous or intraperitoneal routes (Sbrana *et al.*, 2007a; Hutson *et al.*, 2009; Schultz *et al.*, 2009; Stabenow *et al.*, 2010). However, none of these animal models have all aspects of smallpox disease that are observed in humans and there is no aerosol spread of the viral infection between animals. It is highly desirable to have a model of orthopoxvirus infection in which a low dose of aerosolized virus can initiate illness and in which the disease is naturally transmitted from animal to animal by an aerosol route; rabbitpox virus (RPXV) in rabbits meets these criteria.

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Characteristics of RPXV

RPXV is a large, dsDNA virus within the family *Poxviridae* and the genus *Orthopoxvirus*. Although the origin of RPXV is unknown, it has been long suspected that it is a variant of VACV. Recent genome sequencing analyses indicate that the Utrecht strain of RPXV is very closely related to the Copenhagen, Western Reserve and Modified vaccinia virus Ankara (MVA) strains of VACV, and Li *et al.* (2005) concluded that VACV and RPXV might be considered strains of the same virus. However, the RPXV genome has a 719 bp region and three genes that VACV lacks; these genes are associated with virulence of poxviruses and are conserved in VARV (Li *et al.*, 2005).

Rabbitpox outbreaks

Rabbitpox was first described by Greene after three outbreaks in rabbits in the laboratories of the Rockefeller Institute for Medical Research between 1930 and 1933 (Greene, 1933, 1934a, b; Rosahn & Hu, 1935; Rosahn *et al.*, 1936). The disease was very contagious and the mortality rate was high (up to 50 %). Because this disease was caused by a poxvirus and the clinical signs (which included fever, lymphadenitis, pock-like eruption in the skin and mucous membranes, keratitis or ophthalmia and orchitis) were very similar to smallpox in humans, it was named 'rabbitpox'. The last outbreak of rabbitpox at the Rockefeller Institute ended after the imposition of vaccination and strict isolation procedures.

In 1941, there was a report of a similar disease outbreak among rabbits at the University of Utrecht in The Netherlands (Jansen, 1941). However, in contrast to the previous outbreaks at the Rockefeller Institute, lesions in the skin and/or mucous membranes were not a prominent feature of this disease, and when they did occur it was usually late in the disease course; this clinical presentation eventually became known as 'pock-less' rabbitpox (Jansen, 1947). Several subsequent outbreaks of rabbitpox occurred in Europe and the USA, and in all instances the disease resembled that seen at the University of Utrecht (Christensen *et al.*, 1967). Sick rabbits primarily showed respiratory signs and it was thought that the transmission occurred through a respiratory route. This route of transmission was confirmed through experimental transmission studies (Bedson & Duckworth, 1963; Westwood *et al.*, 1966; Adams *et al.*, 2007).

In vitro studies compared an isolate of RPXV from the Rockefeller Institute to an isolate from the University of Utrecht and the results showed that the Utrecht strain was distinct from the Rockefeller strain regarding haemagglutinin activity (Fenner, 1958). All reported rabbitpox outbreaks occurred in laboratory colonies of rabbits; the disease has not been recognized in wild rabbit populations, and there have not been any rabbitpox outbreaks in laboratories reported since the 1960s. Although experimental studies of RPXV continue, these are all done with the Utrecht strain of RPXV; the Rockefeller strain of RPXV apparently no longer exists.

Transmission studies

The 1932 rabbitpox outbreak in the Rockefeller Institute is a good example of how easily RPXV can be naturally transmitted from rabbit to rabbit; within 1 month, more than 1400 rabbits (almost the entire colony) were infected, and this included animals housed in four different rooms and two separate buildings (Greene, 1933, 1934a, 1935). During the terminal stages of this outbreak, Greene performed some simple transmission studies by placing sick rabbits into cages with naïve animals and he determined that the incubation period between exposure and development of clinical signs was 4.5–9 days (Greene, 1934a). However, these experiments did not demonstrate if viral transmission occurred primarily by direct contact or by inhalation of aerosolized viral particles.

Similarly, Bedson and Duckworth performed transmission studies by placing sick rabbits with naïve rabbits and concluded that rabbit-to-rabbit spread only occurs when nasal and ocular discharges are present in infected animals (Bedson & Duckworth, 1963). Subsequently, Westwood *et al.* (1966) demonstrated rabbit-to-rabbit aerosol transmission of RPXV by placing uninfected rabbits in separate cages in the same room with RPXV-exposed rabbits. Recently, Adams *et al.* (2007) performed both contact and aerosol transmission studies and confirmed the results of the older studies.

These studies demonstrated that RPXV could be naturally transmitted between rabbits by direct contact or aerosol. However, multiple other routes have been used experimentally to infect rabbits with RPXV (Table 1). Independent of the route of infection, all rabbits developed a similar lethal systemic disease. The primary difference among these different routes was the minimum dose of virus necessary for infection. While less than 1 p.f.u. caused disease intradermally, larger amounts of virus were required to produce disease by intravascular, intraperitoneal, intranasal or conjunctival routes (Pearce *et al.*, 1936; Bedson & Duckworth, 1963; Christensen *et al.*, 1967). However, infection with aerosolized RPXV is the best way for studying the natural disease in rabbits and also provides an animal model of aerosolized orthopoxvirus infection.

Aerosolized rabbitpox disease

In natural transmission of RPXV, the aerosolized particles are >5 μm in diameter and are deposited in the upper respiratory tract (Thomas, 1970). In experimental aerosolized RPXV transmission studies, the diameter of the mechanically generated aerosol particles is ~1 μm and viral deposition generally occurs in the lower respiratory tract (Westwood *et al.*, 1966). With either natural or experimental aerosol exposure of rabbits to RPXV, the infection progresses in a very similar fashion to smallpox disease in humans (Henderson, 2002); however, the clinical course of the disease in rabbits experimentally infected with aerosolized RPXV tends to progress more rapidly than that in naturally infected rabbits. RPXV first replicates in the respiratory tract and then spreads to regional lymph nodes (e.g. mediastinal and/or mandibular lymph nodes). This is followed by the development of a primary viraemia, resulting in viral seeding of the liver and spleen where secondary replication of the virus occurs (Westwood *et al.*, 1966; Nalca *et al.*, 2008). If this secondary replication is sufficiently high to cause a secondary viraemia, the virus spreads to other internal organs and the skin. Recent studies have shown that clinical signs and disease progression depend on the dose of aerosolized virus (Garza *et al.*, 2009). When rabbits are exposed to a low

Table 1. Results of experimental infection of rabbits with RPXV

Route of infection	Dose	Clinical outcome	Reference(s)
Intravenous	>110 p.f.u.	Conjunctivitis, diarrhoea, mortality	Bedson & Duckworth (1963); Christensen *et al.* (1967)
Intraperitoneal	Unknown*	Conjunctivitis, diarrhoea, mortality	Pearce *et al.* (1936); Christensen *et al.* (1967)
Subcutaneous	6–12 p.f.u.	Oedematous lesion on the injection side, mortality	Christensen *et al.* (1967)
Intranasal	1 000 p.f.u.	Fever, nasal and conjunctival discharges, viraemia, mortality at higher doses	Bedson & Duckworth (1963)
Conjunctival	>10 000 p.f.u.	Fever, viraemia	Bedson & Duckworth (1963)
Intradermal	1–100 p.f.u.	Skin necrosis (inoculation site), viraemia, secondary skin-lesions, mortality	Bedson & Duckworth (1963); Adams *et al.* (2007)
Intratesticular	6–12 p.f.u.	Fever, nasal and conjunctival discharges, acute haemorrhagic orchitis, viraemia, mortality	Pearce *et al.* (1936); Bedson & Duckworth (1963)
Aerosol	15 p.f.u.	Fever, nasal and conjunctival discharges, pneumonia, mortality	Westwood *et al.* (1966); Nalca *et al.* (2008)

*Inoculation dose of virus not calculated.

dose (<200 p.f.u.) of aerosolized RPXV, the resultant disease has several features that were seen in humans with the 'ordinary form' of smallpox (Councilman *et al.*, 1904; Bras, 1952a; Fenner *et al.*, 1988a). With low-dose exposure, the incubation time in rabbits is 4–6 days and fever is the first clinical sign of the disease, followed by anorexia, weakness, rapid weight loss and depression. Lesions on the skin and/or mucous membranes are first noticeable around days 9–10 and typically first appear as small tan to red macules on the lips, nose, ears and eyelids, and then other areas of skin (A. Nalca, unpublished data); the number of such lesions is highly variable and ranges from a few scattered lesions to too many to be counted. Rabbits eventually become lethargic, their body temperature drops to subnormal levels and death usually occurs between 8 and 14 days after exposure. It is worth noting that the rash that develops in rabbits with rabbitpox is somewhat different than what occurs in humans with smallpox. Smallpox-skin lesions begin as macules that progress to papules, vesicules and pustules, which become umbilicated and form scabs that often leave a scar after they are removed (Bras, 1952a, b). The skin lesions in rabbitpox start with macule formation followed by progression to papules, vesicles and pustules. These usually heal without scabbing or scarring and the time period of progression from macule to disappearance is much shorter than with smallpox lesions.

Inoculation of rabbits with a high dose (>200 p.f.u.) of aerosolized RPXV produces an overwhelming and uniformly lethal infection that progresses much more quickly and infected animals develop high viral titres in their blood (Nichols *et al.*, 2006; Garza *et al.*, 2009); this is similar to the 'haemorrhagic form' of smallpox (Fenner *et al.*, 1988a). After 2–3 days incubation, fever develops and clinical signs such as anorexia, weakness, rapid weight loss and depression appear; purulent oculonasal discharge and facial oedema are also marked signs of the disease. Rapid disease progression usually results in death by day 6 post-exposure before development of visible skin lesions. If skin lesions occur, they are typically present on the lips, eyelids and/or areas that were shaved before viral challenge (Chapman *et al.*, 2010). The appearance of cutaneous lesions in these areas is most probably secondary to irritation and localized inflammation; a similar phenomenon occurred in people with smallpox and was called 'the garter effect' (Fenner *et al.*, 1988a). Table 2 provides a comparison between the clinical features of ordinary-type smallpox and rabbitpox at low- and high-aerosol exposures.

Gross and histological lesions

In addition to the skin lesions mentioned above, rabbits also typically have lesions in mucous membranes of the pharynx, larynx, upper trachea and tongue (Greene, 1934b; Nichols *et al.*, 2006); this is similar to smallpox in humans (Bras, 1952a). Histologically, the epidermis of the skin and the mucosal epithelium of the mucous membranes display ballooning degeneration, necrosis and vesicle formation, accompanied by varying degrees of inflammation (Rosahn & Hu, 1935; Bras, 1952a, b; D. K. Nichols, unpublished data).

Testicular lesions frequently occur in humans with smallpox and rabbits with rabbitpox and characteristically consist of foci of interstitial necrosis, haemorrhage and inflammation (Greene, 1934b; Rosahn & Hu, 1935; Bras, 1952a; Lancaster *et al.*, 1966; Nalca *et al.*, 2008). Similar foci of necrosis and inflammation are also often present in the ovaries of affected rabbits (Greene, 1934b; Nalca *et al.*, 2008).

Unlike smallpox in humans, RPXV infections in rabbits usually produce lung lesions that are severe enough to cause death (Lancaster *et al.*, 1966; Nichols *et al.*, 2006; Adams *et al.*, 2007). Grossly, the lungs are diffusely oedematous and may have conical-to-wedge-shaped areas

Table 2. Similarities and differences between smallpox and rabbitpox (aerosol exposure)

	Smallpox (ordinary type)	Rabbitpox (low-dose exposure)	Rabbitpox (high-dose exposure)
Transmission	Aerosol	Aerosol	Aerosol
Incubation period	7–17 days	4–6 days	2–3 days
Prodromal phase	2–4 days	2–4 days	0–2 days
Clinical signs	Fever, oropharyngeal lesions, skin lesions	Fever, oropharyngeal lesions, nasocular discharges, dyspnoea, skin lesions	Fever, oropharyngeal lesions, nasocular discharges, dyspnoea, (+/−) skin lesions
Occurrence of initial skin lesions	~ Day 15	~ Days 9–10	~ Days 6–7
Skin lesion characteristics	Macules→papules→ vesicles→pustules→ scabs→scars	Macules→papules→ vesicles→ pustules	Macules→papules→ (+/−) vesicles
Complications	Pneumonia, blindness, encephalitis	Pneumonia, foci of necrosis and inflammation in multiple organs	Pneumonia, foci of necrosis and inflammation in multiple organs
Case fatality rate	~ 30 %	~ 100 %	100 %
Occurrence of death	~ Days 22–28	~ Days 8–14	~ Days 5–7

of bronchopneumonia (Nichols *et al.*, 2006; Nalca *et al.*, 2008, Garza *et al.*, 2009); however, in a recent publication, the lungs are described as having large areas of haemorrhage rather than pneumonia (Adams *et al.*, 2007). Histologically, there are foci of necrosis and inflammation centred on bronchi and bronchioles, with severe inflammation and degeneration of pulmonary veins, arteries and arterioles (Lancaster *et al.*, 1966; Nichols *et al.*, 2006; Nalca *et al.*, 2008; Garza *et al.*, 2009).

Also in contrast to humans with smallpox, rabbits affected with rabbitpox have foci of necrosis surrounded by infiltrates of inflammatory cells in other organs. These lesions most commonly occur in the liver and adrenal cortex, and less frequently in the spleen, lymph nodes and other organs (Lancaster *et al.*, 1966; Christensen *et al.*, 1967; Nalca *et al.*, 2008).

Testing medical countermeasures

The antiviral drug 'ST-246' (SIGA) has been shown to be very effective against other orthopoxviruses in other animal species (Yang *et al.*, 2005; Quenelle *et al.*, 2007a, b; Sbrana *et al.*, 2007b; Huggins *et al.*, 2009; Jordan *et al.*, 2009), and we demonstrated that it is also highly efficacious in protecting rabbits against a lethal dose of aerosolized RPXV (Nalca *et al.*, 2008). Daily oral administration of ST-246 to rabbits, started 1 h after viral exposure and continued for 14 days, resulted in 100 % survival. Furthermore, the efficacy of delayed drug therapy was evaluated by beginning treatment on days 1, 2, 3 or 4 post-exposure. Although some of the rabbits from the days 1 and 2 post-exposure treatment groups displayed clinical signs of rabbitpox, their illness usually resolved very quickly, and the survival rates for these group of rabbits were 88 and 100 %, respectively. However, when the treatment was initiated on days 3 or 4 post-exposure, survival was 67 and 33 %, respectively.

We also used this model to test the efficacy of a third-generation smallpox vaccine IMVAMUNE (MVA-BN) against aerosolized orthopoxviral infection (Garza *et al.*, 2009). In this study, rabbits were vaccinated once with a low dose of IMVAMUNE or a high dose of IMVAMUNE, or twice (14 days apart) with a high dose of IMVAMUNE. All rabbits were challenged with a lethal dose of aerosolized RPXV 4 weeks after the last vaccination and, although some of the rabbits vaccinated once with low-dose IMVAMUNE showed some signs of the rabbitpox disease, none of these rabbits died. All of the rabbits vaccinated once or twice with high-dose IMVAMUNE were fully protected from clinical disease and death.

Conclusion

Potential disadvantages of using rabbits in research include the paucity of immunological reagents for this species and the lack of immunodeficient and/or gene knockout rabbit strains; this limits some studies of disease pathogenesis and host-immune response. However, in contrast to other animal models of orthopoxvirus infection, aerosolized RPXV in rabbits demonstrates that exposure to low doses of the virus results in generalized viral dissemination, secondary lesions, animal-to-animal transmission and lethality. These are all characteristics of VARV infection in humans which means that this is an excellent animal model for smallpox and for testing the efficacy of medical countermeasures against smallpox. Additional studies to further characterize this rabbitpox model and the disease transmission dynamics are warranted.

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