



Mousepox: A Small Animal Model for Biodefense Research

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Abstract

Ectromelia virus is the causative agent of the disease mousepox. Mousepox is arguably the best small animal model available for the study of smallpox. This model is under utilized due to *ectromelia virus*' potential to cause explosive epizootics in mouse colonies; however, with modern animal husbandry techniques and biocontainment facilities, these concerns are no longer founded. The mousepox model is used to study basic questions in pathogenesis, to evaluate prophylactic and therapeutic treatments for smallpox, and to develop countermeasures to orthopoxviruses bioengineered for enhanced virulence, transmissibility, or the ability to break-through vaccine immunity. Some of the "dual use" knowledge gained from these experiments can be used for the improvement of human welfare or, in the wrong hands, the generation of biological weapons. The Institutional Biosafety Committee will likely become an important component in the evaluation of dual use technology.

Introduction

The poxvirus family is divided into two subfamilies: *Entomopoxvirinae* (poxviruses of insects) and *Chordopoxvirinae* (poxviruses of vertebrates). The *Chordopoxvirinae* are further classified into genera through studies of cross-protection in animals and cross-neutralization in tissue culture. Species of the same genera cross-protected or cross-neutralized each other, and share similar biological properties (Buller & Palumbo, 1991; Moss, 2001). Vaccinia virus

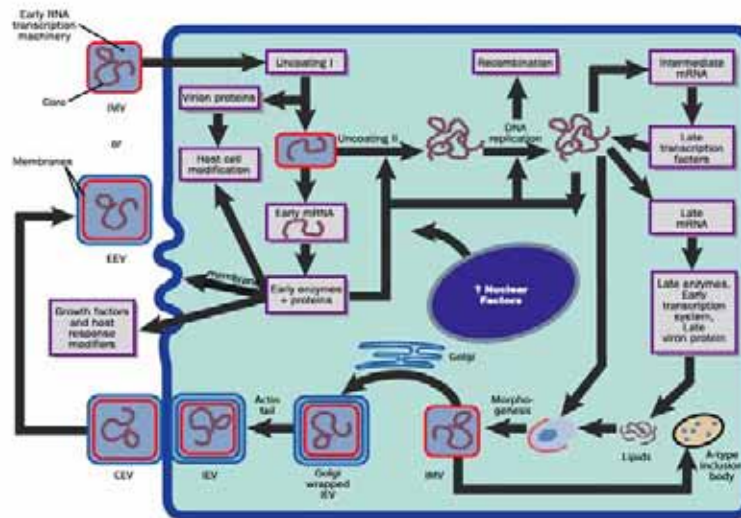
(VACV) is the prototypic member of the orthopoxvirus genus along with closely related cowpox, ectromelia, monkeypox, and variola viruses. Variola virus (VARV) is the causative agent of smallpox.

Poxviruses are the largest of all animal viruses and can be visualized by light microscopy, although the details of the virion structure remain obscure. High resolution electron microscopy shows virions to be oval or brick-shaped structures of about 200 to 400 nm in length with axial ratios of 1.2 to 1.7. Each virion contains one DNA molecule associated with proteins and organized in a nucleoprotein core. One or two lateral bodies appear to be present in the concave region between the core wall and a membrane, which contains a number of virus-encoded proteins.

Orthopoxvirus Replication Cycle

Using mainly the prototypic orthopoxvirus, vaccinia, a large number of investigators over the last 30 years have described in great detail the features of the intracellular replication cycle of orthopoxviruses (Figure 1) (Moss, 2001). The orthopox virion containing early RNA transcription machinery attach to, and fuse with, the plasma membrane. Within 15 minutes the virion transcription machinery is activated (uncoating I). Early genes are expressed that code for a variety of functions that modify the host cell for optimal virus replication, attenuate the host response to infection, and mediate virus synthetic processes. After further uncoating (uncoating II), and between 1.5 to 6 hours post infection, the virus

Figure 1
General orthopoxvirus replication cycle.



IMV, intracellular mature virus; IEV, intracellular enveloped virus; CEV, cell-associated enveloped virus, and EEV, extracellular enveloped virus.

genome is replicated via concatamers. From progeny DNA templates, late transcription factors are expressed from intermediate genes and late gene RNA is synthesized. Late genes encode the early transcription system, enzymes, and structural proteins necessary for virion assembly. By 4 hours post infection virion morphogenesis commences with the formation of membrane structures in the intermediate compartment and the packaging of resolved unit length genomic DNA. The first infectious form of the virus is the intracellular mature virus (IMV), which has one membrane derived from the intermediate compartment. The IMV may remain in the cytoplasm or in certain virus species such as ectromelia (ECTV) and cowpox (CPXV) become occluded in an A-type inclusion body. Some IMV acquire an additional double layer of intracellular membrane derived from the *trans* Golgi network that contains unique virus proteins (intracellular enveloped virus, IEV). The IEV is transported to the periphery of the cell where fusion with the plasma membrane ultimately results in release of extracellular enveloped virus (EEV) or, if attached to the exterior surface of the plasma membrane, remains as cell associated enveloped virus (CEV). While IMV and

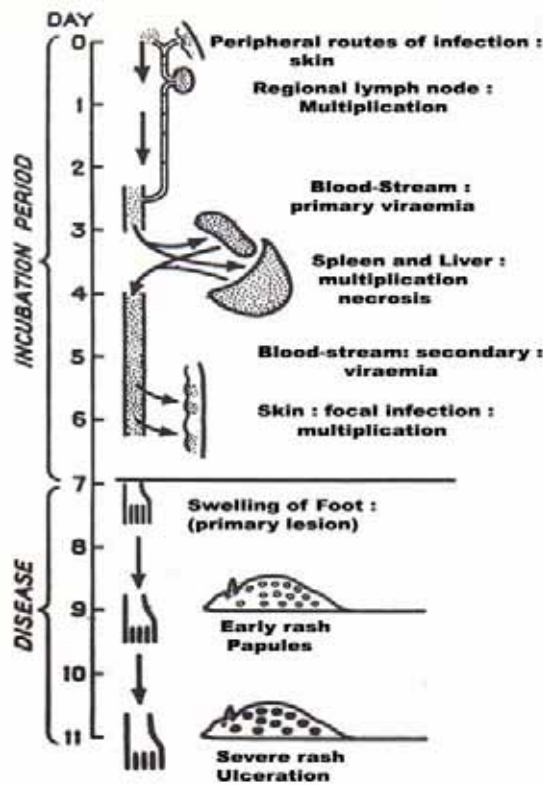
CEV/EEV are infectious, the external antigens on the two virion forms are different, and thus each virion type probably binds to different cellular receptors and are likely uncoated by different mechanisms. EEV is thought to be most important in cell-to-cell spread and systemic disease, whereas the occluded virus in the A-type inclusion body may enhance virion stability in the environment outside the host animal.

Mousepox

Ectromelia virus is the causative agent of mousepox, an acute exanthematous disease of mouse colonies in Europe, Japan, China, and the U.S. (Buller & Palumbo, 1991; Dick et al., 1996; Fenner, 1982). The natural reservoir for ECTV is unknown but one report provides evidence that wild mice may be involved (Fenner, 1982). Laboratory studies have shown ECTV to have a very narrow host range, infecting only certain mouse species (Buller, Potter, & Wallace, 1986; Fenner, 1982). A number of different strains of ECTV have been isolated which have been shown to differ in their virulence for the mouse (Buller & Palumbo, 1991; Fenner, 1982).

Figure 2

Pathogenesis of an intradermal infection of mice with ectromelia virus.



Natural infections with ECTV occur through microscopic abrasions, which allow access of the virus to the epidermal or dermal layers of the cornified mouse skin (Roberts, 1962b). The infection subsequently spreads rapidly along the basal keratinocyte layer above the basement membrane, and in the dermis where all cell types appear to support infection except possible the mast cells (Chen, Buller, Wall & Upton, 2000; Roberts, 1962b). ECTV can be detected in the draining lymph node of susceptible and resistant mice as early as 8 hours p.i. ECTV invasion of internal organs is thought to be by cell-associated virus or free virus via the afferent lymphatics, draining lymph node, and bloodstream (primary viremia) Mims, 1964; Payne & Kristensson, 1985). By 3 days p.i., spleen and liver contain antigen positive cells in discrete foci of coagulative necrosis. By 4 or 5 days p.i., virus is reproducibly detected in blood (secondary viremia), and subsequently is detected as antigen and infectious virus in most tissues examined (bone marrow, intestine, thymus, nasal mucosa, ovary, vagina, uterus, and/or skin) (Fenner, 1948a; Fenner, 1948b; Jacoby & Bhatt, 1987). Virus replication in skin results in the typical exanthem, which can be seen as early as 6 days p.i., and is dependent on the mouse strain. In skin lesions, the virus multiplies in the epidermis, hair follicles, and sweat glands, and when virus infectivity reaches approximately 10^7 particles per g (-day 9), pale, flat crops of lesions (rash) appear and ulcerate within a few days. Virus is transmitted from the primary and secondary lesions. Experiments have shown the infectious period to last from days 6 to 17 p.i., but transmission efficiency varies depending on the virus strain, mouse strain, and route of infection (Wallace & Buller, 1985). Death is observed from 7 to 14 days p.i. depending on multiplicity of infection and mouse and virus strain. Recovery from infection is thought to be due to rapid and strong innate responses to infection including interferons and natural killer cells, cell mediated responses including CTL and $CD4^+$ T cells, and circulating antibody, which can be detected by day 7 and reached a maximum by day 14 (Blanden, 1974; Blanden & Gardner, 1976; Buller, Bhatt, and Wallace, 1983; Fenner, 1948a; Jacoby, Bhatt, & Brownstein, 1989; Karupiah, 1998; Karupiah, Buller, Van Rooijen, Duarte, & Chen, 1996; Karupiah Fredrickson, Holmes, Khairallah & Buller, 1993). (From Fenner, 1948b with permission).

The Moscow, Hampstead, and NIH79 strains are the most thoroughly studied with the Moscow strain being the most infectious and virulent for the mouse. In the late 1940s, mousepox was proposed as a model for the study of the pathogenesis of smallpox and generalized vaccinia in humans (Fenner, 1948b). Studies from a succession of investigators in the last 5 decades have resulted in a detailed description of the virologic and pathologic disease course in genetically susceptible (A, BALB/c, DBA/2, and C3H/He; death -7 days post infection) and resistant (C57BL/6 and AKR) inbred and out-bred mice (Figure 2); identification and characterization of the important cell-mediated and innate responses for recovery from infection; and the discovery of *rmp-1*, *rmp-2*, *rmp-3*, and *rmp-4* loci which govern resistance to severe mousepox (Brownstein, 1998; Brownstein & Gras, 1995; Delano & Brownstein, 1995). Varying mouse genotype, virus strain and dose of virus result in distinct disease patterns for a given route of infection.

Mousepox: A Small Animal Model for Smallpox

Mousepox differs from smallpox in at least two features post respiratory tract infection. First, the disease course in mousepox is shorter as compared

to smallpox. The eclipse period in mousepox and smallpox are 6 and 10 days, respectively. Fatal cases of mousepox usually occur within 7 to 14 days post infection, whereas deaths in ordinary smallpox occur within approximately 18 to 22 days p.i. Second, the major lesions in mousepox are observed in the liver and spleen, whereas these organs are relatively uninvolved in smallpox (Buller, Bhatt, & Wallace, 1983; Fenner, Henderson, Arita, Jezek, & Ladnyj, 1988). A feature of mousepox that is similar to smallpox is the relatively small dose of virus that is required to initiate disease in the upper and lower respiratory tract (Tables 1 and 2). Another similarity is the detection of virus in respiratory gases during the pre-exanthem period (Roberts, 1962a). And finally both diseases present with a characteristic exanthematous rash. In the case of mousepox, the development of a rash is dependent on a number of parameters including mouse strain, virus strain, route of inoculation, and virus dose (Buller & Palumbo, 1991).

Biocontainment of Ectromelia Virus

Ectromelia virus was originally discovered in a mouse colony at a biomedical research institution. In subsequent years it was isolated from a number of mouse colonies in Europe, North America, and Asia. It was not always possible to ascertain the

Table 1
Intranasal Ectromelia Virus Infection of A/NCR mice

Virus Dose (PFU/mouse) ¹	Mean Time to Death	Mortality	LD ₅₀ (PFU)
5,000	7±0.0	100%	
500	7.3±0.5	100%	
50	8±0.0	100%	
5.0	9±0.0	100%	0.3
0.5	10±1.4	50%	
0.05	10	25%	

¹Mice were anesthetized by an intraperitoneal injection of a mixture of ketamine (90 mg/kg)/xylazine (10mg/kg). The mouse was held at a 45° angle and 3-5 µl of virus suspension was dispensed into one of the nares followed by a 10 min incubation to allow for complete aspiration of the drop. A second dose was then applied to the other nares followed by an additional 10 min aspiration period.

means by which the virus was introduced into mouse colonies; however, several routes were likely. The stability of the ectromelia virion suggests that fomites must always be considered as a source of the virus following an epizootic. However, most often the disease outbreak was traced to the importation of an infected mouse into the mouse colony or as a contaminant of cells such as hybridomas that were passaged in mice for ascites production (Buller, Weinblatt, Hamburger, & Wallace, 1987; Fenner, 1982; Wallace et al., 1981). In the 1995 epizootic of mousepox at the Naval Medical Research Institute in Bethesda, Maryland, the source of ECTV was traced to a lot of commercial, pooled mouse sera used to stabilize *Plasmodium yoelii* prior to injection into BALB/cByJ mice (Dick et al., 1996). Modern animal husbandry practices (e.g., quarantine of imported animals and disease surveillance programs) have greatly reduced the incidence of “natural” mousepox. With the availability of modern containment facilities, there is no rational reason for the prohibition of mousepox research providing the appropriate biosafety procedures are followed. The following describes the biocontainment of ECTV research at Saint Louis University.

Research Laboratory

Nonanimal research with ECTV is carried out in a BSL-2 laboratory essentially as described in the 4th edition of *Biosafety in Microbiological and Biomedical Laboratories*. ECTV causes disease in only certain mouse species and does not productively infect rats, hamsters, rabbits, or humans (Buller, Potter, & Wallace, 1986; Fenner, 1982). Thus, vaccination of laboratory personnel with the smallpox vaccine is not a prerequisite for working with this virus. All manipulations with infectious virus are carried out in a recirculating class II biosafety cabinet housed within a separate room within the main laboratory. This room contains the incubators that are used to grow and plaque the virus and is kept locked when not in use. Liquid and dry wastes are autoclaved prior to conventional disposal. Plaque assay plates are treated for 30 minutes with a 25% volume of crystal violet/formalin staining solution (0.13% crystal violet [w/v], 5% ethanol [v/v], and 11% formaldehyde [v/v]) in order to visualize plaques and inactivate

virus. Personal protective equipment consists of laboratory coats and disposable gloves.

Animal Facility

Due to its potential threat to the vivarium’s mouse population, ECTV research involving mice is carried out in the institution’s ABSL-3 facility. Personal protective equipment consists of disposable booties, disposable lab coats, gloves, and surgical masks (optional). Mice are housed in autoclavable cages with attached micro-isolator bonnets and maintained in negative pressure cubicles within the ABSL-3 suite. All husbandry and experimental protocols are carried out in a 6-foot class II biosafety cabinet containing an integral dump station and directly exhausted to the outside through a HEPA filter. Disinfectants Spor-klenz and Expor are used for surface decontamination. Inocula and other materials contaminated with infectious ECTV are transported between the BSL-2 laboratory and ABSL-3 facility in sealed containers such as centrifuge tubes in a Saf-T-Pac product SPP-104.

The Use of the Mousepox Model in Biodefense Research

Basic Studies in Orthopoxvirus Pathogenesis

Since the laboratory mouse is a “natural” host of ECTV, all of the approximately 175 ECTV genes have evolved to function in a “mouse environment” (Chen et al., 2003). This is an important aspect to consider when selecting a model to study pathogenesis as some poxvirus-encoded proteins evolved to modify the hosts’ response to infection. The function of these genes can be divided into broad categories including inhibition of apoptosis, blockade of the inflammatory response, and blockade of the immune response. Some of these interactions can have a high degree of species specificity. For example, one of VARV immunomodulators, smallpox inhibitor of complement enzymes (SPICE), is 100-fold more potent than the homologous inhibitor, vaccinia virus complement-control protein (VCP), at inactivating C3b, and six-fold more potent than VCP at inactivating C4b (Rosengard, Liu, Nie, & Jimenez). Similarly, the ECTV IFN- γ binding pro-

tein (BP) binds mouse IFN- γ , whereas the homologue of the nonmouse pathogen, VACV, does not (Mossman, Upton, Buller, & McFadden, 1995). Since deletion experiments have shown these host response modifiers can have profound effects on virus pathogenesis, it is prudent (if possible) to select an experimental model where the poxvirus naturally infects the host, and one is confident that all of the virus genes are likely functional. Furthermore, ECTV is the only orthopoxvirus where transmission can be easily demonstrated between an index mouse and contacts in a laboratory setting. This allows the use of a transmission assay to evaluate the importance of individual genes or candidate antivirals.

Antiviral and Vaccine Efficacy Testing

An important use of the mousepox model is for evaluation of orthopoxvirus antivirals and vaccines. The mousepox model is arguably the best mouse model available for this purpose. For comparison, the commonly used CPXV mouse model required an aerosol exposure dose of 5×10^6 plaque forming units (PFU) of CPXV to obtain 100% mortality with 3-week-old BALB/c mice, and lowering the exposure dose to 5×10^4 PFU caused only transient, mild illness and weight loss, but no deaths (Bray et al., 2000). In addition, the resistance of mice to CPXV increases with the age of the animal, which makes vaccine challenge studies difficult to carry out. Thus, the ECTV aerosol model provides a much greater dynamic range for evaluating antivirals. One can

choose an aerosol lethal dose of 100 PFU, which is 3-fold greater than the LD₅₀ value of 32 PFU (Table 2), and is likely in the range of the infectious dose for aerosolized smallpox as extrapolated from the Meschede Hospital incident and the 1971 smallpox epidemic in Aralsk, Kazakhstan (Tucker & Zilinskas, in press; Wehrle et al., 1970). Alternatively, one could use a dose 1,000 to 10,000 times the LD₅₀ to fully examine the robustness of the test antiviral.

The Development of Countermeasures to Bioengineered Orthopoxviruses

Historically VARV had a 20% to 40% mortality rate in populations that were partially immune as a consequence of smallpox vaccination and recovery from the natural illness. In naïve populations, the mortality rate could exceed 90% due to the combination of a lack of preexisting immunity and the breakdown of the social structure of the community (Fenner et al., 1988). Although VARV was eradicated in 1979, there is a growing concern that it could be used as a bioweapon by terrorists or a rogue nation(s). In addition, there is a lower, but measurable, risk that VARV may be engineered to make it more virulent and/or transmissible for naïve or vaccinated populations. This possibility was highlighted by a research study carried out by a group of Australian scientists led by Dr. Ron Jackson (Jackson et al., 2001).

The focus of the research of the Jackson group was to develop a method to control frequent mouse

Table 2
Aerosol Ectromelia Virus Infection of A/NCR mice

Presented Dose (PFU/mouse) ¹	Mean Time to Death	Mortality	LD ₅₀ (PFU)
1.9×10^4	8.1±0.4	100%	
1.3×10^3	9.3±0.5	100%	
63	10.2±1.3	67%	32
6.3	14	9%	
0	N/A	0%	

¹Eight mice were exposed to the indicated presented dose of a small particle aerosol in a Nose-only Inhalation Exposure System (CH Technologies, Inc). Mice were observed for a period of 21 days.

epidemics in Australia. Building on the experience of Australian biologists in 1950s who attempted to control the burgeoning feral European rabbit populations with a highly lethal rabbit pathogen, myxoma virus (Fenner & Woodroffe, 1953), Jackson planned to control mouse populations through vector-mediated immuno-contraception using ECTV (Tyndale-Biscoe, 1994). The target antigen was zona pellucida glycoprotein 3 (ZP3), which is a constituent of the matrix surrounding mammalian oocytes, ovulated eggs, and early embryos, and is expressed only in the ovary (Epifano et al., 1995). Zona pellucida glycoprotein 3 has been extensively studied as a target antigen for immuno-contraception, and a number of studies have shown that passive immunization with ZP3 specific monoclonal antibodies or active immunization with ZP3 peptides conferred infertility (East, Gulyas & Dean, 1985; Lou, Ang, McElveen, & Tung, 1995; Millar, Chamow, Baur, Oliver, Robey, & Dean, 1989). In the initial Jackson study, an ECTV recombinant expressing the cDNA for ZP3 was constructed and used to immunize female mice. The immunized mice developed anti-ZP3 antibody titers and were infertile for 5-9 months following immunization. Mice regained fertility as the anti-ZP3 antibody levels decreased, but infertility could be restored with a second immunization with the ECTV recombinant expressing ZP3 (Jackson, Maguire, Hinds, & Ramshaw, 1998). Since the goal of this research was to develop a vector system that would result in life-long infertility following a single infection, a second study sought to increase the magnitude of the anti-ZP3 antibody response following immunization. The chosen approach was to co-express a CD4⁺ Th2 cytokine, IL-4. Unexpectedly, the ECTV recombinant expressing both mouse IL-4 and ZP3 was lethal for the normally resistant C57BL/6 mouse strain and for C57BL/6 mice previously vaccinated with an attenuated ECTV (Jackson et al., 2001). Although the mechanism by which ECTV virulence was enhanced is not clearly understood, it involves an IL-4-mediated immunosuppression of the innate and acquired immune responses to ECTV infection.

These findings were discussed in the editorial columns of a number of scientific journals where the conclusions were extrapolated to the closely re-

lated VARV; speculating that similar approaches could be used to create a super poxvirus that would be vaccine-proof (Dennis, 2001; Finkel, 2001). In lieu of this concern, we have evaluated a number of approaches to prophylactically treat infections of C57BL/6 mice with ECTV expressing mouse IL-4. To date, we have developed several efficacious treatment regimens including combinations of immunization with the smallpox vaccine, antiviral therapy using cidofovir, which targets the DNA polymerase of a number of viruses including poxviruses, and/or immunotherapy with an IL-4 neutralizing monoclonal antibody, 11B11 (data not shown). This work supports the utility of the mousepox model for developing treatment approaches to bioengineered poxviruses such as those expressing immunosuppressive cytokines or toxic molecules.

The Dilemma of “Dual Use” Technology

The biological sciences, in general, and Biodefense Research in particular, are providing technological breakthroughs that can be used for the improvement of human welfare or, in the wrong hands, the generation of biological weapons. The Jackson study is an example of unexpected research findings that could not easily have been predicted at the outset. It underscores the difficulty in regulating studies at their inception that may generate dual use knowledge. Instead, a heightened awareness of the dual use potential of scientific discoveries throughout the scientific process may better suit the post 9/11 world. This could be achieved by the efforts of the scientists carrying out the studies, the Institutional Biosafety Committees (IBC) charged with regulatory compliance, and the peer-review processes responsible for funding the proposals, and dissemination of knowledge in the oral and written media. A recent report by the National Academy of Sciences (*Biotechnology Research in an Age of Terrorism: Confronting the “Dual Use” Dilemma*) emphasizes an important role for the IBC in this process (National Academy of Sciences [in press]). The report recommended that the Recombinant DNA Advisory Committee draft a detailed set of guidelines to facilitate IBC review of seven classes of experiments that could provide dual use technology.

The experiments of concern would:

1. Demonstrate how to render a vaccine ineffective
2. Confer resistance to therapeutically useful antibiotics or antiviral agents
3. Enhance the virulence of a pathogen or render a nonpathogen virulent
4. Increase transmissibility of a pathogen
5. Alter the host range of a pathogen
6. Enable the evasion of diagnostic/detection modalities
7. Enable weaponization of a biological agent or toxin

Under these criteria, an experiment to construct an ECTV expressing an immunosuppressive cytokine (such as mouse IL-4) would trigger an additional level of review that is based on the first and third classes of experiments. This would occur in spite of the fact that ECTV is not a human pathogen, and the ECTV recombinant expressing mouse IL-4 poses absolutely no risk for humans.

Acknowledgments

Robert Mark Buller was supported by a National Institute of Allergy and Infectious Diseases (NIAID) contract NO1-AI-15436. The content of this article does not necessarily reflect the policy of the Federal government. We would like to thank Monica Allen for editorial assistance and Ms. Jill Schriewer and Gelita Owens for technical expertise. We would like to also thank Dr. Richard Doyle and Mr. Mark Campbell for critically reading this manuscript.

References

- Blanden, R. V. (1974). T cell response to viral and bacterial infection. *Transplantation Reviews*, *19*, 56-88.
- Blanden, R. V., & Gardner, I. D. (1976). The cell-mediated immune response to ectromelia virus infection. I. Kinetics and characteristics of the primary effector T cell response in vivo. *Cellular Immunology*, *22*, 271-282.
- Bray, M., Martinez, M., Smee, D. F., Kefauver, D., Thompson, E., & Huggins, J. W. (2000). Cidofovir protects mice against lethal aerosol or intranasal cowpox virus challenge. *Journal of Infectious Diseases*, *181*, 10-19.
- Brownstein, D. G. (1998). Comparative genetics of resistance to viruses. *American Journal of Human Genetics*, *62*, 211-214.
- Brownstein, D. G., & Gras, L. (1995). Chromosome mapping of Rmp-4, a gonad-dependent gene encoding host resistance to mousepox. *Journal of Virology*, *69*, 6958-6964.
- Buller, R. M., Bhatt, P. N., & Wallace, G. D. (1983). Evaluation of an enzyme-linked immunosorbent assay for the detection of ectromelia (mousepox) antibody. *Journal of Clinical Microbiology*, *18*, 1220-1225.
- Buller, R. M., & Palumbo, G. J. (1991). Poxvirus pathogenesis. *Microbiological Reviews*, *55*, 80-122.
- Buller, R. M., Potter, M., & Wallace, G. D. (1986). Variable resistance to ectromelia (mousepox) virus among genera of Mus. *Current Topics in Microbiology & Immunology*, *127*, 319-322.
- Buller, R. M., Weinblatt, A. C., Hamburger, A. W., & Wallace, G. D. (1987). Observations on the replication of ectromelia virus in mouse-derived cell lines: Implications for epidemiology of mousepox. *Laboratory Animal Science*, *37*, 28-32.
- Chen, N., Buller, R. M., Wall, E. M., & Upton, C. (2000). Analysis of host response modifier ORFs of ectromelia virus, the causative agent of mousepox. *Virus Research*, *66*, 155-173.
- Chen, N., Danila, M. I, Feng, Z., Buller, R. M., Wang, C., Han, X., Lefkowitz, E. J., & Upton, C. (2003). The genomic sequence of ectromelia virus, the causative agent of mousepox. *Virology*, *317*, 165-186.
- Delano, M. L., & Brownstein, D. G. (1995). Innate resistance to lethal mousepox is genetically linked to the NK gene complex on chromosome 6 and correlates with early restriction of virus replication by cells

- with an NK phenotype. *Journal of Virology*, 69, 5875-5877.
- Dennis, C. (2001). The bugs of war. *Nature*, 411, 232-235.
- Dick, E. J., Jr., Kittell, C. L., Meyer, H., Farrar, P. L., Ropp, S. L., Esposito, J. J., Buller, R. M., Neubauer, H., Kang, Y. H., & McKee, A. E. (1996). Mousepox outbreak in a laboratory mouse colony. *Laboratory Animal Science*, 46, 602-611.
- East, I. J., Gulyas, B. J., & Dean, J. (1985). Monoclonal antibodies to the murine zona pellucida protein with sperm receptor activity: effects on fertilization and early development. *Developmental Biology*, 109, 268-273.
- Epifano, O., Liang, L. F., Familari, M, Moos, M. C. Jr., & Dean, J. (1995). Coordinate expression of the three zona pellucida genes during mouse oogenesis. *Development*, 121, 1947-1956.
- Fenner, F. (1948a). The clinical features and pathogenesis of mouse-pox (infectious ectromelia of mice). *The Journal of Pathology and Bacteriology*, 60, 529-551.
- Fenner, F. (1948b). The pathogenesis of the acute exanthems. An interpretation based on experimental investigations with mousepox (infectious ectromelia of mice). *The Lancet*, ii, 915-920.
- Fenner, F. (1982). Mousepox. In H. L. Foster, J. D. Small & J. G. Fox (Ed.). *The mouse in biomedical research*. New York: Academic Press.
- Fenner, F., Henderson, D. A., Arita, I., Jezek, Z., & Ladnyj, I. D. (1988). *Smallpox and its eradication*. Geneva: World Health Organization.
- Fenner, F., & Woodroffe, G. M. (1953). The pathogenesis of infectious myxomatosis: the mechanism of infection and the immunological response in the European rabbit (*Oryctolagus cuniculus*). *British Journal of Experimental Pathology*, 34, 400-411.
- Finkel, E. (2001). Engineered mouse virus spurs bioweapons fears. *Science*, 291, 585-585.
- Jackson, R. J., Maguire, D. J., Hinds, L. A., & Ramshaw, I. A. (1998). Infertility in mice induced by a recombinant ectromelia virus expressing mouse zona pellucida glycoprotein 3. *Biological Reproduction*, 58, 152-159.
- Jackson, R. J., Ramsay, A. J., Christensen, C. D., Beaton, S., Hall, D. F., & Ramshaw, I. A. (2001). Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox. *Journal of Virology*, 75, 1205-1210.
- Jacoby, R. O., & Bhatt, P. N. (1987). Mousepox in inbred mice innately resistant or susceptible to lethal infection with ectromelia virus. II. Pathogenesis. *Laboratory Animal Science*, 37, 16-22.
- Jacoby, R. O., Bhatt, P. N., & Brownstein, D. G. (1989). Evidence that NK cells and interferon are required for genetic resistance to lethal infection with ectromelia virus. *Archives of Virology*, 108, 49-58.
- Karupiah, G. (1998). Type 1 and type 2 cytokines in antiviral defense. *Veterinary Immunology & Immunopathology*, 63, 105-109.
- Karupiah, G., Buller, R. M., Van Rooijen, N., Duarte, C. J., & Chen, J. (1996). Different roles for CD4+ and CD8+ T lymphocytes and macrophage subsets in the control of a generalized virus infection. *Journal of Virology*, 70, 8301-8309.
- Karupiah, G., Fredrickson, T. N., Holmes, K. L., Khairallah, L. H., & Buller, R. M. (1993). Importance of interferons in recovery from mousepox. *Journal of Virology*, 67, 4214-4226.
- Lou, Y., Ang, J., Thai, H., McElveen, F., & Tung, K. S. (1995). A zona pellucida 3 peptide vaccine induces antibodies and reversible infertility without ovarian pathology. *Journal of Immunology*, 155, 2715-2720.

- Millar, S. E., Chamow, S. M., Baur, A. W., Oliver, C., Robey, F., & Dean, J. (1989). Vaccination with a synthetic zona pellucida peptide produces long-term contraception in female mice. *Science*, *246*, 935-938.
- Mims, C. A. (1964). Aspects of the pathogenesis of virus diseases. *Bacteriological Reviews*, *28*, 30-71.
- Moss, B. (2001). Poxviridae: The viruses and their replication. In D. M. Knipe & P. M. Howley (Eds.), *Fields Virology Volume 2*, (pp. 2849-2883). New York: Lippincott Williams and Wilkins.
- Mossman, K., Upton, C., Buller, R. M., & McFadden, G. (1995). Species specificity of ectromelia virus and vaccinia virus interferon-gamma binding proteins. *Virology*, *208*, 762-769.
- National Academy of Sciences. (In Press). *BioTechnology Research in an age of terrorism: Confronting the "dual use" dilemma*. Washington, DC: National Academies Press.
- Payne, L. G., & Kristensson, K. (1985). Extracellular release of enveloped vaccinia virus from mouse nasal epithelial cells in vivo. *Journal of General Virology*, *66*, 643-646.
- Roberts, J. A. (1962a). Histopathogenesis of mousepox. I. Respiratory infection. *British Journal of Experimental Pathology*, *43*, 451-461.
- Roberts, J. A. (1962b). Histopathogenesis of mousepox. II. Cutaneous infection. *British Journal of Experimental Pathology*, *43*, 462-468.
- Rosengard, A. M., Liu, Y., Nie, Z., & Jimenez, R. (2002). Variola virus immune evasion design: expression of a highly efficient inhibitor of human complement. *Proceedings of the National Academy of Sciences, USA*, *99*, 8808-8813.
- Tucker, J. B., & Zilinskas, R. A. (in press). *The 1971 smallpox epidemic in Aralsk, Kazakhstan, and the Soviet biological warfare program*. Monterey, CA: Center for Nonproliferation Studies.
- Tyndale-Biscoe, C. H. (1994). Virus-vectored immunocontraception of feral mammals. *Reproduction, Fertility and Development*, *6*, 281-287.
- Wallace, G. D., & Buller, R. M. (1985). Kinetics of ectromelia virus (mousepox) transmission and clinical response in C57BL/6j, BALB/cByj and AKR/J inbred mice. *Laboratory Animal Science*, *35*, 41-46.
- Wallace, G. D., Werner, R. M., Golway, P. L., Hernandez, P. L., Alling, D. W., & George, D. A. (1981). Epizootiology of an outbreak of mousepox at the National Institutes of Health. *Laboratory Animal Science*, *31*, 1-15.
- Wehrle, P. F., Posch, J., Richter, K. H., & Henderson, D. A. (1970). An airborne outbreak of smallpox in a German hospital and its significance with respect to other recent outbreaks in Europe. *Bulletin of the World Health Organization*, *43*, 669-679.