Implications of rodent viral infections for research

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Introduction

Rodents are susceptible to an array of viral infections many of which spread rapidly because of the population density and turnover typically encountered in a vivarium. The effects of infection in individual animals are, in turn, influenced by host-related factors such as age, genetic susceptibility and immune status and by virus-related factors such as virulence, stability and tropism. Virus – host interactions produce infections that range from lethal to asymptomatic and from acute to persistent. The impact of severe infections is usually apparent, but the effects of asymptomatic infections, that are detected only by seroconversion, are harder to gauge. They will depend on the research for which the affected animals will be used, the harm the causative agents may pose to other animals or to animal products such as cell lines and transplantable tumors and, in the case of zoonotic agents, the risk to laboratory workers. The research scientist and the vivarium director are challenged to assess correctly the risks from adventitious infection for the welfare of the staff, the animals and the research.

Because of the myriad ways in which laboratory rodents are used, intuitive research on interference from viruses may lead to unproductive "fishing expeditions". Few studies have dealt specifically with effects of rodent viruses on research, documented interference under conditions that mimic infection or, in the case of older studies, established that the animals employed were free of adventious infection. Therefore clues to interference must often be infected from studies of pathogenesis and epizootiology or from unexpected research results associated with infection. Either source may be useful to guide further experimentation.

This brief review focuses on conventional viruses that may interfere with research employing common laboratory rodents: mice, rats, hamsters and guinea pigs. They are separated by nucleic acid type and then by virus or virus group. We attempt to highlight conditions one is likely to encounter during natural rather than experimental infection. Extensive reviews of rodent viral infections, including their potential for interference, are available elsewhere. Among the most useful of these are: *Infectious Diseases of Mice and Rats* (*Lindsey et al.* 1991), *Viral and Mycoplasmal Infections of Laboratory Rodents: Effects on Biomedical Research* (*Bhatt et al.* 1986), *Complications of Viral and Mycoplasmal Infections in Rodents to Toxicology Research and Testing* (*Hamm* 1985) and a series of texts on the biology and diseases of laboratory animals developed by the American College of Laboratory Animal Medicine (*Foster et al.* 1982, *Lindsey et al.* 1979, *van Hoosier & MacPherson* 1987, *Wagner & Manning* 1976). We have, however, included some citations from recent research that is not addressed in these texts.

DNA viruses

Ectromelia virus

Ectromelia virus is an *Orthopoxvirus* of mice that is antigenically related to vaccinia virus. It is thought to be transmitted primarily through skin wounds, but airborne transmission cannot be ruled out. Viral replication at the site of entry extends to regional lymph nodes and is followed by viremia. Genetically susceptible mice develop lethal, fulminant infection with severe necrosis especially in lymphoid, hematopoietic and alimentary tissues. Moderately resistant mice may survive, but they can develop ex-

anthemas from which the infection derives its common name, mousepox. The skin lesions contain infectious virus and contribute to protracted environmental contamination with virus. Genetically resistant mice often have asymptomatic infection, but may also shed virus during early stages and put susceptible mice at risk. Infection is diagnosed from seroconversion, clinical signs, and lesions that include necrosis and intracytoplasmic inclusions. Apart from the exanthematous form, infection normally lasts less than three to four weeks.

Research complications stem from pathogenic infections where morbidity and mortality are high. Mice and mouse products obtained from countries where the virus is endemic should be quarantined and evaluated before use.

Rodent adenoviruses

There are two known mouse adenoviruses designated MAd-1 and MAd-2, (formerly strains FL and K-87), respectively. Antibodies also occur in rats, but the eliciting agent(s) have not been isolated. Serological studies in the United States indicate that natural infection is caused by MAd-2. It is usually asymptomatic and spreads by the oral-fecal route. Viral replication in intestinal epithelium is expressed by intranuclear inclusions. MAd-1 can cause fatal multisystemic necrosis after experimental inoculation of infant mice.

Interference from MAd/2 infection has not been reported. MAd-1, apart from inducing fatal infection in infant mice, is reported to render the kidneys of adult mice more susceptible to bacterial infection.

Lethal interstitial pneumonia accompanied by adenoviral inclusions has been reported in guinea pigs (*Kunstyr et al.* 1984). The causative agent has not been isolated and specific serological assays are not available, but the agent appears to share antigens with MAd-2.

Rodent parvoviruses

Minute virus of mice (MVM) and two viruses of rats, rat virus (RV) and H-1 virus, are well-recognized rodent parvoviruses. Aditional viruses have recently been found in mice and there is serological evidence for corresponding agents in rats and hamsters. They are currently called rodent orphan parvoviruses (OPVs) to imply rudimentary understanding of their biology (*McKisic et al.* 1993, *Smith et al.* 1993). The rodent parvoviruses are antigenically related but can be distinguished by serological tests that detect virus-specific proteins. Natural infection, however, appears to be species-specific and is often asymptomatic. The pathogenicity of parvoviruses is episodic and stems from cytolytic replication in cells transiting the S phase of the mitotic cycle. Thus parvoviruses are more pathogenic for very young rodents than for adults.

RV in particular is more pathogenic than the other rodent parvoviruses. Lethal infections can develop *in utero* and infants may die from multisystemic necrosis that is commonly prevalent in the liver and cerebellum. Hemorrhagic infarcts, primarily of the central nervous system, can develop in infants and weanlings.

Parvoviruses are excreted in feces, urine and probably by the respiratory tract, although routes of excretion may vary. They are transmitted by animal contract and fomites, but airborne transmission may be important. Infection in immunocompetent adults elicits seroconversion and is often acute and self-limiting. Athymic adults and euthymic infants, by contrast, may develop persistent RV infection (*Gaertner et al.* 1989, *Jacoby et al.* 1991). OPV appears to persist in mice infected as infants or adults for at least several weeks after seroconversion. Persistent infections have not been demonstrated for MVM. Maternal antibody protects infant rats from RV infection, but immune defences in parvovirus infections are generally not well-defined.

Potential complications from parvoviruses are inferred from their predilection for dividing cells and their tropism for endothelium, hematopoietic tissue and lymphoreticular tissues (*Gaertner et al.* 1992). In addition to overt disease, concern should focus on research where cell replication is critical such as immunology and oncology. Immunosuppression has been demonstrated for mouse and rat parvoviruses. However, the full immunomodulatory and oncolytic potential of parvoviruses is unclear. Conversely, research that induces immunosuppression or tissue damage may "activate" silent parvovirus infection which, in the case of RV, may have disruptive or lethal effects. Parvoviruses also commonly contaminate transplantable tumors and cell cultures.

Rodent herpesviruses

Rodent herpesviruses have been studied extensively as models for human infection, but natural infections are infrequent. Species-specific cytomegaloviruses are known for mice, rats and guinea pigs. Mice have a second herpesvirus, mouse thymic virus, and two additional herpesviruses have been isolated from guinea pigs. None of these agents cause clinical disease during natural infection and only one, mouse thymic virus, causes a significant lesion, thymic atrophy in neonatally infected mice. They are, however, transiently immunosuppressive under experimental conditions and, at least one, mouse cytomegalovirus, may enhance susceptibility to bacterial infection. Furthermore they can persist after the onset of humoral immunity. Chronic excretion, especially in the saliva of persistently infected animals, leads to contact infections that could be troublesome in breeding colonies or among immunodeficient animals.

RNA viruses

Sendai virus

Sendai virus is a *Paramyxovirus* that causes pneumonia in mice, rats, hamsters, guinea pigs and other rodents. Clinical disease is most frequent in mice. Infant, immunodeficient and genetically susceptible mice are at risk for severe or lethal pneumonia and convalescence in enfants is often accompanied by retarded growth. Immunodeficient animals also may develop persistent pneumonia and wasting. Infection usually begins in the upper respiratory tract and proceeds to bronchiolitis and alveolitis within 10 days. Recovery is accompanied by seroconversion and repair of the lung with varying degrees of scarring. The duration of immunity is unknown and susceptibility to re-infection has not been studied. Lesions in rats resemble those in mice, but clinical signs are uncommon. Hamsters and guinea pigs also are prone to silent infection.

Interference from Sendai virus infection stems primarily from pneumonia. Affected mice endure anesthesia poorly and reparative scarring may interfere with pulmonary physiology research. Mice may also have increased susceptibility to bacterial infection including mycoplasmosis and to lung neoplasia from chemical carcinogens. Immunomodulatory effects have been described in rats and mice, but apart from inhibition of lung macrophages, there is little evidence for direct effects of virus on lymphoreticular cells. The stress of acute infection may provoke transient immunosuppression or other stress-related phenomena such as fetal resorption.

Pneumonia Virus of Mice (PVM)

PVM is a *Pneumovirus* that infects mice, rats, hamsters, guinea pigs and possibly other laboratory rodents. Infection is typically asymptomatic and expressed as mild rhinitis and interstitial pneumonia. The course is acute and accompanied by seroconversion, but persistent pneumonia and wasting has been reported in immunodeficient mice. Little is known about interference from PVM infection, but the potential to compromice anesthesia and facilitate opportunistic pathogens should be considered (*Roths et al.* 1993).

Mouse coronaviruses

Mouse coronaviruses are commonly grouped as mouse hepatitis virus, a term that is misleading for at least two reasons. First, numerous strains of mouse coronavirus exist in nature and they differ in virulence, tissue tropism and antigenic profile. Second, hepatitis is an uncommon feature of natural infection although it was often encountered in experimental infection, especially after parenteral inoculation. These viruses are, however, among the most common infectious agents of laboratory mice and have a significant potential to interfere with research.

Natural infection is limited to mice and tends to be polytropic (*Compton et al.* 1993). Polytropic strains initiate respiratory infection followed by broad dissemination that often involves lymphoreticular tissues. Encephalitis occurs occasionally by direct extension of infection from the nose to the brain. Enterotropic strains colonize the intestine and, in infant mice, can cause fatal enteritis or enterotyphlocolitis. Infection in immunocompetent adult mice is often asymptomatic and generally lasts less than a month. Recovery is accompanied by seroconversion and lesions may be limited to the development of virus antigen-positive syncytia in the alimentary tract, lymphoid tissue, vascular endothelium or elsewhere. Infection in immunodeficient animals, by contrast can be chronic and debilitating. The proclivity of coronaviruses for antigenic variation implies that immunity to one strain does not ensure immunity to other strains. Thus enzootic infection may reflect sequential exposure to related, but antigenically distinct strains of virus rather than the result of persistent infection with one strain.

Mouse coronaviruses can distort research with mice in various ways ranging from the induction of severe clinical disease to the alteration of serum biochemistry. Immune dysfunction, the expression and duration of which may be difficult to predict, is among the most troublesome effects and a refelection of viral lymphocytotropism (*Cook-Mills et al.* 1992, *de Souza & Smith* 1991, *Smith et al.* 1991). Coronaviruses can also contaminate cell lines and transplantable neoplasms including hybridomas. Because these viruses are ubiquitous and their effects can be unpredictable, the potential for interference should be explored if research results are unexpectedly distorted in a setting where active infection is suspected.

Rat coronaviruses

As for mouse coronaviruses, multiple strains of rat coronaviruses have been isolated for which rats appear to be the only natural host. Infection is highly prevalent and is rapidly transmitted by aerosol among rats of all ages. It begins as acute rhinitis followed by mild tracheobronchitis and alveolitis. Virus also attacks serous and mixed salivary glands and lacrimal glands causing necrotizing sialoadenitis and dacryoadenitis. Clinical signs include sneezing, photophobia, lacrimation, chromodacryorrhea and cervical swelling. Infection is acute and seroconversion is usually followed by complete recovery. Immunity is longlived, but transient re-infection can occur as can mild infection with heterologous strains of virus (*Percy et al.* 1990, *Weir et al.* 1990a). Athymic rats develop protracted sialodacryoadenitis and interstitial pneumonitis with spread of virus to atypical sites like urinary mucosa (*Weir et al.* 1990b).

Complications from rat coronavirus infection include increased risk from anesthesia due to respiratory inflammation, inappetence and transient effects on reproductive efficiency. Susceptibility to mycoplasmosis, and potentially to opportunistic organisms is increased. Dacryoadenitis can lead to acute or chronic keratoconjunctivitis, accompanied occasionally by other severe manifestations of eye disease such as synechia, hypopyon, hyphema and secondary glaucoma.

Mouse encephalomyelitis virus

Mouse encephalomyelitis virus is a picornavirus of mice although seroconversion has

been detected occassionally in rats. Natural infection is usually enteric and asymptomatic, but occassional extension to the central nervous system may cause posterior paralysis from poliomyelitis. Laboratory strains can produce acute, lethal encephalitis or immunemediated demyelination after parenteral inoculation. Infection is often acute and accompanied by seroconversion, but chronic infection with viral excretion may occur in seropositive animals (*Brownstein et al.* 1989).
Interference with research is likely to be minimal apart from the risk of neurological involvement especially where host defences or alimentary barriers to infection are compromised.

Reoviruses

The tree mammalian reovirus serotypes (1, 2 and 3) possess distinguishable hemagglutinins. Reoviruses commonly infect mice, but rats, hamsters, guinea pigs and man are susceptible. Reovirus 3 is often assumed to be the sole cause of infection in laboratory rodents, but this assumption may be unwarranted since generic serological tests that are often used for health monitoring do not distinguish the 3 serotypes. Infection in adult mice is asymptomatic, but diarrheal disease has been reported in sucklings. Experimental infection of 2 day old mice recently showed that virulence is virus strain-dependent and can range from mild, transient enteritis for reovirus 2 to hepatoencephalitis, myocarditis and lymphoid depletion for reoviruses 2 and 3 (*Barthold et al.* 1993). Infection typically lasts 3 weeks or less and is transmitted by the oral-fecal route.
The mild, transient nature of reovirus infection makes it a minor nuisance in most research settings. Reoviruses are, however, common contaminants of cell lines and transplantable tumors. Experimental infection has been reported to suppress carcinogenesis and enhance tumor immunity.

Rotaviruses

Rotaviruses are Reoviridae that commonly infect many mammals including man. Two antigenically distinguishable rotaviruses are known to infect laboratory rodents: mouse rotavirus (epidemic diarrhea of infant mice (EDIM) virus) and rat rotavirus (infectious diarrhea of infant rats (IDIR) virus or rat rotavirus-like agent). Both cause diarrhea in infant animals with atendant weight loss and runting. Although morbidity may be high, mortality is usually low, in part because animals continue to nurse, and complete recovery is common. Lesions are restricted to intestinal villus epithelium which can be vacuolated, clubbed and attenuated, although stress may provoke thymic atrophy. Immunocompetent adults develop only asymptomatic infection.
Interference with research stems from transient diarrhea and runting. An agent similar to rat rotavirus has been identified in human patients and raises the possibility of cross-infection.

Lymphocytic choriomeningitis virus (LCMV)

LCMV is an *Arenavirus* that has been used extensively to investigate immune defences against viral infections and viral-induced immunological injury. While its value for immunological research is beyond question, LCMV must be treated with caution as it is pathogenic for man, causing a flu-like illness that may progress to fatal encephalitis. Natural rodent hosts include mice, hamsters, and guinea pigs, but rats and other species can be infected by inoculation. Transmission occurs by contact with contaminated urine, saliva or milk as well as transplacentally. Infection of infant or adult mice can be acute and uneventful, but more complicated sequelae are often encountered. Persistent infection with viraemia and viruria results from *in utero* or neonatal exposure of mice and in hamsters infected at any age. Clinical signs are uncommon during natural infec-

tion, but several forms of LCMV disease have been characterized, especially in mice. Acute infections in infant mice can be fatal and acute infections in adults can provoke lethal T cell-mediated immunological injury the distribution of which depends on viral tropism. Neurotropic strains can provoke lethal lymphocytic choriomeningitis from which the virus gets its name, whereas viscerotropic strains commonly cause necrosis and inflammation in the liver. Lymphocytolysis is also associated with acute infection. Persistent infection leads to immune complex disease marked by vasculitis and fatal glomerulonephritis.

Interference from LCMV is significant because of its effects on the immune system and its zoonotic potential. Moreover its ability to persistently infect animals, cell lines and transplantable tumors can lead to the silent spread of infection in laboratories and vivariums. The immunomodulatory effects of LCMV are varied and often hard to predict. Depending on virus-host interactions, LCMV may enhance or suppress immunity. Moreover it can interfere with viral oncogenesis and with the growth of transplantable tumors. There is also evidence of its potential to interfere with "luxury" functions such as the production of pituitary hormones (*Oldstone et al.* 1982, *Klavinskis & Oldstone* 1988). Human exposure most often results from contact with persistently infected hamsters that may excrete large quantities of virus in urine for many months. Mice are less threathening, but immunocompromised mice have been incriminated in human exposure (*Dykewicz* 1992).

Lactic dehydrogenase-elevating virus (LDV)

LDV is a virus of mice that is currently undergoing reclassification. Like LCMV, it can result in lifelong viremic infection with immune complex formation. Natural infection is rare and transmission occurs primarily by inoculation of contaminated cells or tumors. Infection is generally asymptomatic, but is associated with marked elevations of serum enzymes, notably lactic dehydrogenase, due to viral interference with enzyme catabolism by macrophages. Interference from LDV stems primarily from its replication in macrophages. Resultant dysfunctions in immune response will vary depending on mouse strain and age, the timing of virus inoculation and the response being measured. LDV, LCMV, mouse coronaviruses, and perhaps parvoviruses have the potential to mimic each other in their effects on immunity and tumorigenesis. Thus aberrant research results should stimulate diagnostic testing to differentiate these infections.

Hantaviruses

Hantaviruses infect small rodents, including laboratory rats, persistently and asymptomatically. They are the causative agents of hemorrhagic fevers in people and fatal laboratory exposures have been reported from East Asia. The viruses can be transmitted by aerosol and may be carried by animals or by biologicals such as transplantable tumors. Laboratory rats and wild-trapped rodents from enzootic regions such as Asia should be quarantined and tested before use. Monitoring of tumor cell lines is also recommended.

Miscellaneous viruses

We have not included discussion of tumor viruses such as the rodent leukemia viruses, mouse mammary tumor viruses and mouse polyoma virus. They can clearly interfere with research by causing cancer. The reader is referred to other sources such as those listed at the beginning of this article. It is worth adding, however, that hamsters are susceptible to a transmissible lymphoma whose expression is associated with a papovavirus and a retroviral-like agent (*Barthold et al.* 1987).

Finally, we acknowledge K virus, an endotheliotropic papovavirus that can cause interstitial pneumonia and persistent infection of immunodeficient or immunologically im-

mature mice. It is rarely encountered in laboratory mice, but the natural reservoirs appear to be feral and wild mice.

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References
Lindsey JR et al. (eds.): Infectious diseases of mice and rats. National Academy of Sciences, Washington, DC, 1991.
Bhatt PN (eds.): Viral and mycoplasmal infections of laboratory rodents: Effects on biomedical research. Academic Press, Orlando, Florida, 1986.
Hamm TE, Jr (ed.): Complications of viral and mycoplasmal infections in rodents to toxicology research and testing. Hemisphere Press, Washington, DC, 1985.
Foster HL et al. (eds.): The mouse in biomedical research. Academic Press, Orlando, Florida, 1982.
Lindsey JR et al. (eds.): The laboratory rat. Academic Press, New York, 1979.
van Hoosier GL & CW MacPherson (eds.): Laboratory hamsters. Academic Press, Orlando, Florida, 1987.
Wagner JE & PJ Manning: The biology of the guinea pig. Academic Press, New York, 1976.
Kunstyr I et al.: Adenovirus pneumonia in guinea pigs: An experimental reproduction of the disease. Lab. Anim. 1984, *18,* 55–60.
McKisic MD et al.: Identification and propagation of a putative immunosuppressive orphan parvovirus in cloned T cells. J. Immunol. 1993, in press.
Smith AL et al.: In vivo studies of an "orphan parvovirus" of mice. Lab. Anim. Sci. 1993, in press.
Gaertner DJ et al.: Persistence of rat parvovirus in athymic rats. Arch. Virol. 1989, *105,* 259–268.
Jacoby RO et al.: Persistent rat parvovirus infection in individually housed rats. Arch. Virol. 1991, *117,* 193–205.
Gaertner DJ et al.: Characterization of acute rat parvovirus infection by *in situ* hybridization. Virus Res. 1993, in press.
Roths JB et al.: Lethal exacerbation of *Pneumo cystis carinii* Pneumonia (PCP) in *scid* mice after infection by pneumonia virus of mice (PVM). J. Exp. Med. 1993 in press.
Compton SR et al.: Cellular and molecular pathogenesis of coronaviruses. Lab. Anim. Sci. 1993, in press.
Cook-Mills JM et al.: Mouse hepatitis virus suppresses modulation of mouse spleen T cell activation. Immunol. 1992, *75,* 542–545.
de Souza MS & AL Smith: Characterization of accessory cell function during acute infection of BALB/cByJ mice with mouse hepatitis virus, strain JHM. Lab. Anim. Sci. 1991, *41,* 112–118.
Smith AL et al.: In Vitro splenic Tcell responses to diverse mouse genotypes after oronasal exposure to mouse hepatitis virus, strain JHM. Lab. Anim. Sci. 1991, *41,* 106–111.
Percy DH et al.: Duration of protection from reinfection following exposure to sialodacryoadenitis virus in Wistar rats. Lab. Anim. Sci. 1990, *40,* 144–149.
Weir EC et al.: Infection of SDAV-immune rats with SDAV and rat coronavirus. Lab. Anim. Sci. 1990a, *40,* 363–366.
Weir EC et al.: Persistence of sialodacryoadenitis virus in athymic rats. Lab. Anim. Sci. 1990b, *40,* 138–143.
Brownstein DG et al.: Duration and patterns of transmission of Theiler's mouse encephalomyelitis virus infection. Lab. Anim. Sci. 1989, *39,* 299–301.
Barthold SW et al.: Infectivity, disease patterns and serologic profiles of reoviruses serotypes 1, 2, and 3 in infant and weanling mice. Lab. Anim. Sci. 1993, in press.
Oldstone MBA et al.: Virus-induced alterations in homeostasis: Alterations in the differentiated functions of infected cells *in vitro.* Science 1982, *218,* 1125–1127.
Klavinskis LS & MBA Oldstone: Lymphocytic choriomeningitis virus selectively alters differentiated but not housekeeping functions: Block in expression of growth hormone gene is at the level of transcriptional initiation. Virol. 1989, *168,* 232–235.
Dykewicz CA et al.: Lymphocytic choriomeningitis outbreak associated with nude mice in a research institute. J. Am. Med. Assoc. 1992, *267,* 1349–1353.
Barthold SW et al.: Further evidence for papovavirus as the probable etiology of transmissible lumphoma of Syrian hamsters. Lab. Anim. Sci. 1987, *37,* 283–288.