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CHAPTER 23

Viral Infections

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Introduction

In interpreting the microbiological status of laboratory animals, it must be understood that infection and disease are not synonymous. Infection refers to the invasion and multiplication of microorganisms in body tissues and may occur with or without apparent disease. Disease refers to interruption or deviation from normal structure and function of any tissue, organ, or system. Many of the infections with which we are concerned may not cause discernable disease in many strains of mice. However, they may cause inapparent or subclinical changes that can interfere with research. Such interference often remains undetected, and therefore modified results may be obtained and published.

The types of interference of an agent with experimental results may be diverse. There is no doubt that research complications due to overt infectious disease are significant and that animals with clinical signs of disease should not be used for scientific experiments. But also clinically inapparent infections may have severe effects on animal experiments. There are numerous examples of influences of microorganisms on host physiology and hence of the interference of inapparent infections with the results of animal experiments. Many microorganisms have the potential to induce activation or suppression of the immune system or both at the same time but on different parts of the immune system, regardless of the level of pathogenicity. All infections, apparent or inapparent, are likely to increase inter-individual variability and hence result in increased numbers of animals necessary to obtain reliable results. Microorganisms, in particular viruses, present in an animal may contaminate biological materials such as sera, cells, or tumours (Collins and Parker, 1972; Nicklas *et al.*, 1993). This may interfere with *in vitro* experiments conducted with such materials and may also lead to contamination of animals (Lipman *et al.*, 2000b). Mouse antibody production (MAP) testing or

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polymerase chain reaction (PCR) testing of biologics to be inoculated into mice is an important component of a disease prevention programme. Finally, latent infections may be activated by environmental factors, by experimental procedures, or by the combination and interaction between various microorganisms. For all these reasons, prevention of infection, not merely prevention of clinical disease, is essential.

Unfortunately, research complications due to infectious agents are usually considered artefacts and published only exceptionally. Information on influences of microorganisms on experiments is scattered in diverse scientific journals, and many articles are difficult to detect. To address this problem, several congresses were held on viral complications on research. The knowledge available was summarized in conference proceedings (Melby and Balk, 1983; Bhatt *et al.*, 1986b; Hamm, 1986) and has later repeatedly been reviewed (Lussier, 1988; National Research Council, 1991; Baker, 1998; Nicklas *et al.*, 1999).

This chapter covers only viral infections of laboratory mice. Viral infections of mice have been studied in detail, and comprehensive information on their pathogenic potential, their impact on research, and the influence of host factors such as age, genotype, and immune status on the response to infection is available. Bacterial agents may be similarly important, but with few exceptions (e.g. *Helicobacter* species) little is known about their potential to influence host physiology and experiments. Even less is known about most parasites in this regard. Among fungal agents, only *Pneumocystis carinii* can be expected to play a significant role in contemporary mouse colonies. The nomenclature and taxonomy of viruses described are based on recent nomenclature rules by the International Union of Microbiological Societies (2000) and the Universal Virus Database of the International Committee on the Taxonomy of Viruses (http://www.ictvdb.iacr.ac.uk). Retroviruses are not covered in this chapter because they are not included in routine health surveillance programmes and cannot be eradicated with presently available methods. This is because most of them are incorporated in the mouse genome as proviruses and thus are transmitted via germline.

The ability to accurately determine whether or not laboratory animals or animal populations have been infected with virus depends on the specificity and sensitivity of the detection methods used. Most viral infections in immunocompetent mice are acute or short-term, and lesions are often subtle or subclinical. The absence of clinical disease and pathological changes has therefore only limited diagnostic value. However, clinical signs, altered behaviour, or lesions may be the first indicator of an infection and often provide clues for further investigations.

Serology is the primary means of testing mouse colonies for exposure to viruses, largely because serological tests are sensitive and specific, are relatively inexpensive, and allow screening for a multitude of agents with one serum sample. They are also employed to monitor biological materials for viral contamination using the MAP test. Serological tests detect specific antibodies, usually immunoglobulin G (IgG), produced by the host against the virus and do not actually test for the presence of virus. An animal may have been infected, mounted an effective antibody response, and cleared the virus, but remains seropositive for weeks or months or forever, even though it is no longer infected or shedding the agent. Active infection can only be detected by using direct diagnostic methods such as virus isolation, electron microscopy, or PCR. Meanwhile, PCR assays have been established for the detection of almost every agent of interest. They are highly sensitive and depending on the demands, they can be designed to broadly detect all members of a genus or only one species. However, good timing and selection of the appropriate specimen is critical for establishing the diagnosis. In practice, combinations of diagnostic tests are often necessary including the use of sentinel animals or immunosuppression to get clear aetiological results or to avoid consequences from false-positive results.

Reports on the prevalence of viral infections in laboratory mice throughout the world have been published frequently. In general, the microbiological quality of laboratory mice has constantly improved during the last decades, and several agents (e.g. herpes- and polyomaviruses) have been essentially eliminated from contemporary colonies due to advances in diagnostic methodologies and modern husbandry and rederivation practices (Jacoby and Lindsey, 1998; Zenner and Regnault, 2000; Livingston and Riley, 2003). They may, however, reappear, since most have been retained or are still being used experimentally. Furthermore, the general trend towards better microbiological quality is challenged by the increasing reliance of biomedical research on genetically modified and immunodeficient mice, whose responses to infection and disease can be unpredictable. Increasing numbers of scientists are creating genetically modified mice, with minimal or no awareness of infectious disease issues. As a consequence, they are more frequently infected than 'standard' strains of mice coming from commercial breeders, and available information on their health status is often

insufficient. Frequently, they are exchanged between laboratories, which amplifies the risk of introducing infections from a range of animal facilities. Breeding cessation strategies that have been reported to eliminate viruses from immunocompetent mouse colonies may prove to be costly and ineffective in genetically modified colonies of uncertain or incompetent immune status. It must also be expected that new agents will be detected, although only occasionally. Infections therefore remain a threat to biomedical research, and users of laboratory mice must be cognizant of infectious agents and the complications they can cause.

DNA viruses

Herpesviruses

Two members of the family Herpesviridae can infect mice (*Mus musculus*). Mouse cytomegalovirus 1 (MCMV-1) or murid herpesvirus 1 (MuHV-1) belongs to the subfamily Betaherpesvirinae, genus Muromegalovirus. Murid herpesvirus 3 (MuHV-3) or mouse thymic virus (MTV) has not yet been assigned to a genus within the family Herpesviridae. Both viruses are enveloped, double-stranded DNA viruses that are highly host-specific and relatively unstable to environmental conditions such as heat and acidic pH. Both agents are antigenically distinct and do not cross-react in serological tests, but their epidemiology is similar (Cross *et al.*, 1979).

Seropositivity to MCMV-1 was reported in less than 5% of specified pathogen-free (SPF) mouse colonies in the USA in 1996 (Jacoby and Lindsey, 1998), and some institutions reported to have mice 'on campus' that were positive for MTV. In a more recent study, a low rate (0.1%) of samples was found to be positive for MCMV-1 whereas no sample tested positive for MTV (Livingston and Riley, 2003). The data available suggest that the prevalence of both viruses in contemporary colonies and thus their importance for laboratory mice is negligible. However, both MCMV-1 and MTV are frequently found in wild mice, which may be coinfected with both viruses (National Research Council, 1991; Singleton *et al.*, 2000).

MCMV-1 or MuHV-1

Natural infection with MCMV-1 causes subclinical salivary gland infection in mice. The virus persists in the salivary glands (particularly in the submaxillary glands) and also in other organs (Osborn, 1982; Kercher and Mitchell, 2002; Lenzo *et al.*, 2002).

Most information concerning the pathogenesis of MCMV-1 infection is based on experimental infection studies. These results are very difficult to summarize because the outcome of experimental infection in laboratory mice depends on various factors such as mouse strain and age, virus strain and passage history, virus dose, and route of inoculation (Osborn, 1982). In general, newborn mice are most susceptible to clinical disease and to lethal infection. Virus replication is observed in newborn mice in many tissues (for details, see Osborn, 1982) and appears in the salivary glands towards the end of the first week of infection when virus concentrations in liver and spleen have already declined. Resistance develops rapidly after weaning between days 21 and 28 of age. Experimental infection of adult mice results in mortality only in susceptible strains and only if high doses are administered. Not even intravenous or intraperitoneal injections of adult mice usually produce signs of illness in resistant strains (Shanley *et al.*, 1993). Mice of the *H-2*^{b} (e.g. C57BL/6) and *H-2*^{d} (e.g. BALB/c) **haplotype** are more sensitive to experimental infection than are mice of the *H-2*^{k} haplotype (e.g. C3H), which are approximately 10-fold more resistant to mortality than are those of the *b* or *d* haplotype (Osborn, 1986).

Subclinical or latent infections can be activated by immunosuppression (e.g. with cyclophosphamide or cortisone). Reactivation of MCMV-1 occurs also after implantation of latently infected salivary glands into *Prkdc*^{scid} mice (Schmader *et al.*, 1995). Immunodeficient mice lacking functional T cells or natural killer (NK) cells, such as *Foxn1*^{nu} and *Lyst*^{bg} mice, are more susceptible than are immunocompetent animals. Experimental infection in *Prkdc*^{scid} mice causes severe disease or is lethal, with necrosis in spleen, liver, and other organs, and multinucleate syncytia with inclusion bodies in the liver (Reynolds *et al.*, 1993). Similar to AIDS patients infected with human cytomegalovirus, athymic *Foxn1*^{nu} mice experimentally infected with MCMV also develop adrenal necrosis (Shanley and Pesanti, 1986). The virus also replicates in the lungs leading to pneumonitis whereas in heterozygous (*Foxn1*^{nu}/$^{+}$) littermates replication and disease are not seen (Shanley *et al.*, 1997).

The most prominent histological finding of cytomegaloviruses is enlarged cells (cytomegaly) of salivary gland epithelium with eosinophilic nuclear and cytoplasmic inclusion bodies. The inclusion bodies contain viral material and occur in other organs such as

liver, spleen, ovary, and pancreas (Osborn, 1982). Depending on inoculation route, dose, strain, and age of mice, experimental infections may result in inflammation or cytomegaly with inclusion bodies in a variety of tissues, pneumonitis, myocarditis, meningoencephalitis, or splenic necrosis in susceptible strains (National Research Council, 1991; Osborn, 1982; Percy and Barthold, 2001).

Virus is transmitted oronasally by direct contact and is excreted in saliva, tears, and urine for several months. Wild mice serve as a natural reservoir for infection. The virus is most frequently transmitted horizontally through mouse-to-mouse contact but does not easily spread between cages.

It is generally assumed that MCMV-1 has a very low prevalence in contemporary colonies of laboratory mice. The risk of introduction into facilities housing laboratory mice is very low if wild mice are strictly excluded. Monitoring is necessary if populations of laboratory mice may have been contaminated by contact with wild mice. As for other viruses, enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA) are the most appropriate tests for detecting antibodies. As the virus persists, direct demonstration of MCMV-1 in infected mice is possible by PCR (Palmon *et al.*, 2000) or by virus isolation using mouse embryo fibroblasts (3T3 cells).

Although MCMV-1 does not play a significant role as a natural pathogen of laboratory mice, it is frequently used as a model for human cytomegalovirus infection (Bolger *et al.*, 1999). However, the virus is known to influence immune reactions in infected mice and may therefore have impact on immunological research (Osborn, 1986; National Research Council, 1991; Baker, 1998).

MTV or MuHV-3

Mouse thymic virus was detected during studies in which samples from mice were passaged in newborn mice. Unlike other herpesviruses, the virus can not yet be cultured *in vitro* and is propagated by intraperitoneal infection of newborn mice. The thymus is removed 7–10 days later, and thymus suspensions serve as virus material for further studies. The prevalence of MTV is believed to be low in laboratory mice, and for this reason and also due to the difficulties in virus production for serological assays, it is not included in many standard diagnostic or surveillance testing protocols. Limited data are available indicating that it is common in wild mice, and it is also found in laboratory mice (Osborn, 1982; Morse, 1987; National Research Council, 1991). Further, MTV obviously represents a significant source of contamination of MCMV-1 (and vice versa) if virus is prepared from salivary glands since both viruses cause chronic or persistent salivary gland infections and can coinfect the same host.

All mouse strains are susceptible to infection, but natural or experimental infection of adult mice is subclinical. Gross lesions appear only in the thymus and only if experimental infection occurs at an age of less than about 5 days. Virus is present in the thymus but may also be found in the blood and in salivary glands of surviving animals. Salivary glands are the only site yielding positive virus isolations if animals are infected as adults. Mouse thymic virus also establishes a persistent infection in athymic *Foxn1*^{nu} mice, but virus shedding is reduced compared to euthymic mice and virus recovery is possible only in a lower percentage of mice (Morse, 1988).

Pathological changes caused by MTV occur in the thymus, and reduced thymus mass due to necrosis in suckling mice is the most characteristic gross lesion (Percy and Barthold, 2001). Lymphoid necrosis also may occur in lymph nodes and spleen (Wood *et al.*, 1981), with necrosis and recovery similar to that in the thymus. In mice infected during the first 3 days after birth, necrosis of thymus becomes evident within 3–5 days, and its size and weight are markedly reduced at day 12–14. Intranuclear inclusions may be present in thymocytes between days 10–14 post infection. The thymus and the affected peripheral tissues regenerate within 8 weeks after infection. Regardless of the age of mice at infection, a persistent infection is established in the salivary glands, and infected animals shed virus for life.

Several alterations of immune responses are associated with neonatal MTV infection. There is transient immunosuppression, attributable to lytic infection of T lymphocytes, but activity (e.g. response of spleen cells to T cell mitogens) returns to normal as the histological repair progresses (Wood *et al.*, 1981). Selective depletion of $CD4^+$ T cells by MTV results in autoimmune disease (Morse and Valinsky, 1989; Morse *et al.*, 1999). Information about additional influences on the immune system is given by Osborn (1982), National Research Council (1991), and Baker (1998).

In experimentally infected newborn mice, oral and intraperitoneal infections similarly result in thymus necrosis, **seroconversion**, and virus shedding suggesting that the oral–nasal route is likely to be involved in natural transmission (Morse, 1989). The virus spreads to cage mates after long periods of contact. It is transmitted between mice kept in close contact, and transmissibility from cage to cage seems to be low. Mouse thymic

virus is not transmitted to foetuses by the transplacental route, and intravenous infection of pregnant mice does not lead to congenital damage, impairment in size or development, or abortion (St-Pierre *et al.*, 1987).

Mouse thymic virus and MCMV-1 do not cross-react serologically (Cross *et al.*, 1979). Serological monitoring of mouse populations for antibodies to MTV is possible by IFA testing, which is commercially available; ELISA tests have also been established (Morse, 1990b). ELISA and complement fixation yield similar results (Lussier *et al.*, 1988). It must be noted that the immune response depends on the age at infection. Antibody responses are not detectable in mice infected as newborns whereas adult mice develop high titres that are detectable by serological testing. If neonatal infection is suspected, homogenates of salivary glands or other materials can be inoculated into pathogen-free newborn mice followed by gross and histological examination of thymus, lymph nodes, and spleens for lymphoid necrosis (Morse, 1987). Alternatives to the *in vivo* infectivity assay for detecting MTV in infected tissues include a competition ELISA (Prattis and Morse, 1990) and MAP testing, although this is slightly less sensitive than infectivity assays (Morse, 1990a).

Very little experience exists on eradication methods for MTV due to its low prevalence in contemporary mouse colonies. Methods that eliminate other herpesviruses likely will eliminate MTV. Procurement of animals of known negative MTV status is an appropriate strategy to prevent infection. Strict separation of laboratory mice from wild rodents is essential to avoid introduction into laboratory animal facilities.

Mousepox (ectromelia) virus

Mousepox (ectromelia) virus (ECTV) is a member of the genus Orthopoxvirus belonging to the family Poxviridae. It is antigenically and morphologically very similar to vaccinia virus and other orthopoxviruses. Poxviruses are the largest and most complex of all viruses with a diameter of 200 nm and a length of 250–300 nm. Mousepox (ectromelia) virus contains one molecule of double-stranded DNA with a total genome length of 185,000 nucleotides. It is the causative agent of mousepox, a generalized disease in mice. Experimental transmission to young rats (up to 30 days of age) is possible (Jandasek, 1968; Buller *et al.*, 1986).

The virus is resistant to desiccation, dry heat, and many disinfectants. It is not consistently inactivated in serum heated 30 min at 56°C (Lipman *et al.*, 2000b) and persists for 26 weeks when maintained at 4°C in foetal bovine serum (Bhatt and Jacoby, 1987a). Effective disinfectants include vapour-phase formaldehyde, sodium hypochlorite, and iodophores (Small and New, 1981; National Research Council, 1991).

Historically, ECTV has been an extremely important natural pathogen of laboratory mice. The virus was widespread in mouse colonies worldwide and can still be found in several countries. Between 1950 and 1980 almost 40 individual ectromelia outbreaks were reported in the USA. The last major epizootic in the USA occurred in 1979–80 and has been described in great detail (e.g. Wagner and Daynes, 1981). Severe outbreaks were also described in various European countries (Deerberg *et al.*, 1973; Owen *et al.*, 1975; Osterhaus *et al.*, 1981). A more recent outbreak in the USA, which resulted in the eradication of almost 5000 mice in one institution, was described by Dick *et al.* (1996). The most recent well-documented case of mousepox was published by Lipman *et al.* (2000b). Few additional but unpublished cases of ectromelia have been observed thereafter. In a recent survey conducted in the USA, one population was reported to be seropositive for mousepox (Jacoby and Lindsey, 1998).

Natural infections manifest differently depending on many factors. Mousepox may occur as a rapidly spreading outbreak with acute disease and deaths, or may be inconspicuous with slow spreading and mild clinical signs. The mortality rate can be very low in populations in which the virus has been present for long periods. The infection usually takes one of three clinical courses: acute asymptomatic infection, acute lethal infection (systemic form), or subacute to chronic infection (cutaneous form; Fenner, 1981, 1982; Manning and Frisk, 1981; National Research Council, 1991; Dick *et al.*, 1996). The systemic or visceral form is characterized clinically by facial oedema, conjunctivitis, multisystemic necrosis, and usually high mortality. This form is less contagious than the cutaneous form because the animals die before there is virus shedding. The cutaneous form is characterized by typical dermal lesions and variable mortality. The outcome of infection depends on many factors including strain and dose of virus; route of viral entry; strain, age, and sex of mouse; husbandry methods; and duration of infection in the colony. While all mouse strains seem to be susceptible to infection with ECTV, clinical signs and mortality are strain-dependent (Fenner, 1982; Wallace and Buller, 1985, 1986; Brownstein *et al.*, 1989b). Acute lethal (systemic) infection occurs in highly susceptible inbred strains such as DBA/1, DBA/2, BALB/c, A, and C3H/HeJ. Immunodeficient mice

may also be very susceptible (Allen *et al.*, 1981). Outbreaks among susceptible mice can be explosive, with variable morbidity and high mortality (>80%). Clinical disease may not be evident in resistant strains such as C57BL/6 and AKR, and the virus can be endemic in a population for long periods before being recognized. Furthermore, females seem to be more resistant to disease than males, at least in certain strains of mice (Wallace *et al.*, 1985; Brownstein *et al.*, 1989b).

The mechanisms determining resistance versus susceptibility are not fully understood but appear to reflect the action of multiple genes. The genetic loci considered to be important include *H-2D*^{b} (termed *Rmp-3*, resistance to mousepox, on chromosome 17; O'Neill *et al.*, 1983), the *C5* genes (*Rmp-2*, on chromosome 2), *Rmp-1*, localized to a region on chromosome 6 encoding the NK cell receptor NKR-P1 **alloantigens** (Brownstein and Gras, 1997), the *nitric oxide synthase 2* locus on chromosome 11 (Karupiah *et al.*, 1998), and the *signal transducer and activator of transcription 6* locus on chromosome 10 (Mahalingam *et al.*, 2001). Clearance of the virus by the immune system is absolutely dependent upon the effector functions of CD8^{+} T cells while NK cells, CD4^{+} T cells, and macrophages are necessary for the generation of an optimal response (Niemialtowski *et al.*, 1994; Delano and Brownstein, 1995; Karupiah *et al.*, 1996).

Mousepox (ectromelia) virus usually enters the host through the skin with local replication and extension to regional lymph nodes (Fenner, 1981, 1982; Wallace and Buller, 1986; National Research Council, 1991). It escapes into the blood (primary viraemia) and infects splenic and hepatic macrophages resulting in necrosis of these organs and a massive secondary viraemia. This sequence takes approximately 1 week. Many animals die at the end of this stage without premonitory signs of illness; others develop varying clinical signs including ruffled fur, hunched posture, swelling of the face or extremities, conjunctivitis, and skin lesions (papules, erosions, or encrustations mainly on ears, feet, and tail; Figure 23.1). Necrotic amputation of limbs and tails can sometimes be seen in mice that survive the acute phase, hence the original name of the disease '**ectromelia**' (meaning absent or short limbs; Figure 23.2).

Common gross lesions of acute mousepox include enlarged lymph nodes, Peyer's patches, spleen, and liver; multifocal to semiconfluent white foci of necrosis in the spleen and liver; and haemorrhage into the small intestinal lumen (Allen *et al.*, 1981; Fenner, 1982; Dick *et al.*, 1996; Percy and Barthold, 2001). In animals that survive, necrosis and scarring of the spleen can produce a mosaic pattern of white and red-brown areas that is a striking gross finding.

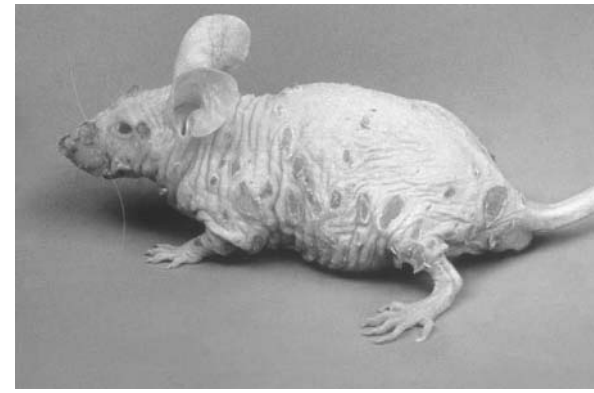

Figure 23.1 The rash of mousepox in a hairless (*hr*) mutant mouse (from Deerberg *et al.*, 1973; used with permission from Verlag M. & H. Schaper).

Figure 23.2 Dry gangrene of the left hind foot of a mouse infected with ECTV.

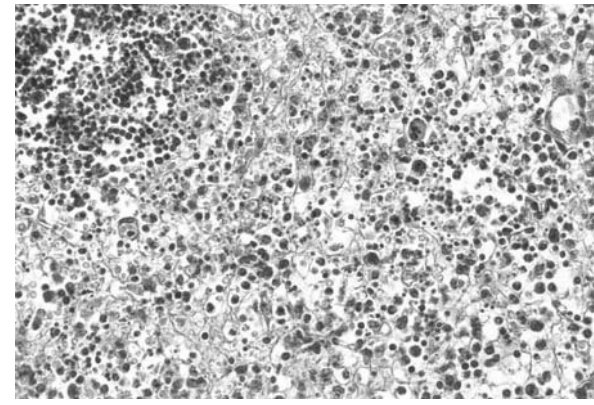

Figure 23.3 Section of the spleen of a mouse infected with ectromelia virus. There is marked parenchymal necrosis with extensive cellular debris and only few lymphoid cells left (H&E stain, magnification 200×; courtesy of Dr. A. D. Gruber).

The most consistent histological lesions of acute mousepox are necroses of the spleen (Figure 23.3),

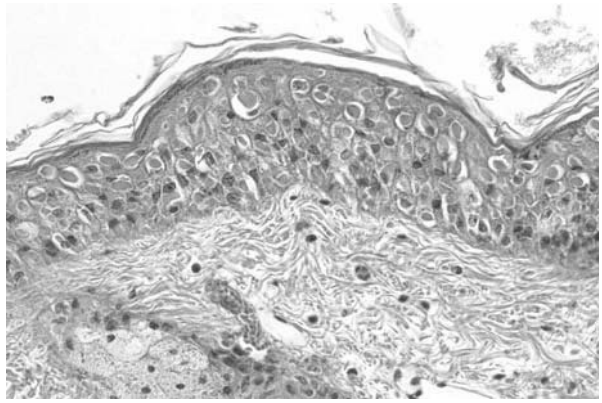

Figure 23.4 Section of the skin of a mouse infected with ECTV. Cutaneous hyperplasia with epithelial cell degeneration and numerous large intraepithelial cytoplasmic viral inclusion bodies (Cowdry type A) are seen (H&E stain, magnification 400 ×; courtesy of Dr. A. D. Gruber).

lymph nodes, Peyer's patches, thymus, and liver (Allen *et al.*, 1981; Fenner, 1982; Dick *et al.*, 1996; Lipman *et al.*, 2000b; Percy and Barthold, 2001). Occasionally, necrosis may also be observed in other organs such as ovaries, uterus, vagina, intestine, and lungs. The primary skin lesion, which occurs about a week after exposure at the site of inoculation (frequently on the head), is a localized swelling that enlarges from inflammatory oedema. Necrosis of dermal epithelium provokes a surface scab and heals as a deep, hairless scar. Secondary skin lesions (rash) develop 2–3 days later as the result of viraemia (Figure 23.1). They are often multiple and widespread and can be associated with conjunctivitis. The skin lesions also can ulcerate and scab before scarring. Mucosal and dermal epithelial cells may have characteristic intracytoplasmic eosinophilic (Cowdry type A) inclusion bodies (Figure 23.4). Basophilic (Cowdry type B) inclusions may be found in the cytoplasm of all infected cells, especially in hepatocytes.

Natural transmission of ECTV mainly occurs by direct contact and **fomites** (Fenner, 1981; Wallace and Buller, 1986; National Research Council, 1991). The primary route of infection is through skin abrasions. Faecal–oral and aerosol routes may also be involved (Werner, 1982). In addition, the common practice of cannibalism by mice may contribute to the oral route of infection (Bhatt and Jacoby, 1987b). Intrauterine transmission is possible at least under experimental conditions (Schwanzer *et al.*, 1975). Virus particles are shed from infected mice (mainly via scabs and/or faeces) for about 3–4 weeks, even though the virus can persist for months in the spleen of an occasional mouse (Bhatt and Jacoby, 1987b; National Research Council, 1991). Cage-to-cage transmission of ECTV and transmission between rooms or units is usually low and largely depends on husbandry practices (e.g. mixing mice from different cages). Importantly, the virus may not be transmitted effectively to sentinel mice exposed to dirty bedding (Lipman *et al.*, 2000b).

Various tests have been applied for the diagnosis of ectromelia. Previous epidemics were difficult to deal with because of limited published data and information on the biology of the virus and the lack of specific and sensitive assays (Wallace, 1981). In the 1950s, diagnosis relied on clinical signs, histopathology, and animal passages of tissues from moribund and dead animals. Culture of the virus on the chorioallantoic membrane of embryonated eggs was also applied. Serology is currently the primary means of testing mouse colonies for exposure to ECTV. The methods of choice are ELISA and IFA; they are more sensitive and specific than the previously used haemagglutination inhibition (HI) assay (Collins *et al.*, 1981; Buller *et al.*, 1983; ACLAD, 1991). Both tests detect antibodies to orthopoxviruses and do not distinguish between ECTV and vaccinia virus. Vaccinia virus is commonly used as antigen for serological testing to avoid the risk of infection for mice. Thus, false-positive serological reactions may be found after experimental administration of replication-competent vaccinia virus. It has been shown that even cage contact sentinels may develop antibodies, and vaccinia virus leading to seroconversion may even be transmitted by dirty bedding (Gaertner *et al.*, 2003). Confirmation of positive serological results is important before action is taken because vaccinia virus is increasingly prevalent in animal facilities as a research tool (e.g. for vaccination or gene therapy). As observed in different outbreaks, serological testing is of little value in the initial stages of the disease. For example, in the outbreak described by Dick *et al.* (1996) depopulation was nearly completed before serological confirmation was possible. For this reason, negative serological results should be confirmed by direct detection methods (PCR, immunohistochemistry, virus isolation) or by histopathology, especially when clinical cases suggestive of mousepox are observed. Polymerase chain reaction assays to detect different genes of poxviruses in infected tissues have been described by Dick *et al.* (1996), Neubauer *et al.* (1997), and Lipman *et al.* (2000b).

The key to prevention and control of mousepox is early detection of infected mice and contaminated biological materials. All institutions that must introduce mice from other than commercial barrier facilities

should have a health surveillance programme and test incoming mice. Perhaps even more important than living animals are samples from mice (tumours, sera, tissues). The virus replicates in lymphoma and hybridoma cell lines (Buller *et al.*, 1987), and such cells or material derived from them may therefore be a vehicle for inadvertent transfer between laboratories. The last two published outbreaks of ectromelia were both introduced into the facilities by mouse serum (Dick *et al.*, 1996; Lipman *et al.*, 2000b). Lipman *et al.* (2000b) found that the contaminated serum originated from a pooled lot of 431 that had been imported from China. Because mouse serum commonly is sold to the end user in small aliquots (few millilitres), it has to be expected that aliquots of the contaminated lot are still stored in numerous freezers. Both cases provide excellent examples of why MAP or PCR testing should be performed on all biological materials to be inoculated into mice.

Eradication of mousepox usually has been accomplished by elimination of the affected colonies, disinfection of rooms and equipment, and disposal of all infected tissues and sera. While culling of entire mouse colonies is the safest method for eradication of mousepox, it is not a satisfactory method due to the uniqueness of numerous lines of genetically modified animals housed in many facilities. Several studies indicate that mousepox is not highly contagious (Wallace and Buller, 1985; Bhatt and Jacoby 1987a,b) and that it may be self-limiting when adequate husbandry methods are applied. Therefore, strict quarantine procedures along with cessation of breeding (to permit resolution of infection) and frequent monitoring with removal of clinically sick and seropositive animals are a potential alternative. The period from the last births before the break until the first matings after the break should be at least 6 weeks (Bhatt and Jacoby, 1987b). Sequential testing of immunocompetent contact sentinels for seroconversion should be employed with this option.

In the past, immunization with live vaccinia virus was used to suppress clinical expression of mousepox. Vaccination may substantially reduce the mortality rate, but it does not prevent virus transmission or eradicate the agent from a population (Buller and Wallace, 1985; Bhatt and Jacoby, 1987c). After vaccination, typical pocks develop at the vaccination site, and infectious vaccinia virus is detectable in spleen, liver, lungs, and thymus (Jacoby *et al.*, 1983). Vaccination also causes seroconversion so that serological tests are not applicable for health surveillance in vaccinated populations. It is therefore more prudent to control mousepox by quarantine and serological surveillance than by relying on vaccination.

Mortality and clinical disease are the major factors by which ECTV interferes with research. Severe disruption of research can also occur when drastic measures are taken to control the infection. The loss of time, animals, and financial resources can be substantial.

Murine adenoviruses

Murine adenoviruses (MAdV) are non-enveloped, double-stranded DNA viruses of the family Adenoviridae, genus Mastadenovirus. Two distinct strains have been isolated from mice. The FL strain (MAdV-1) was first isolated in the USA as a contaminant of a Friend leukaemia (Hartley and Rowe, 1960); the K87 strain (MAdV-2) was first isolated in Japan from the faeces of a healthy mouse (Hashimoto *et al.*, 1966). Both strains are now considered to represent different species (Hamelin and Lussier, 1988; Jacques *et al.*, 1994a,b). In laboratory mice, seropositivity to adenoviruses was reported in 2% of SPF colonies and in 8% of non-SPF colonies in the USA (Jacoby and Lindsey, 1998). Antibodies were also detected at a low prevalence rate in French colonies (Zenner and Regnault, 2000), but the virus strain used as antigen is not mentioned. A similar range of positive samples was reported by Livingston and Riley (2003). Antibodies to MAdV were also found in wild mice (Smith *et al.*, 1993b) and in rats (Otten and Tennant, 1982; Smith *et al.*, 1986).

Both viruses are not known to cause clinical disease in naturally infected, immunocompetent mice. However, MAdV-1 can cause a fatal systemic disease in suckling mice after experimental inoculation (Hartley and Rowe, 1960; Heck *et al.*, 1972; Wigand, 1980). Disease is characterized by scruffiness, lethargy, stunted growth, and often death within 10 days. Experimental infection of adult mice with MAdV-1 is most often subclinical and persistent (Richter, 1986) but can cause fatal haemorrhagic encephalomyelitis with neurological symptoms, including tremors, seizures, ataxia, and paralysis, in susceptible C57BL/6 and DBA/2J mice (Guida *et al.*, 1995). BALB/c mice are relatively resistant to this condition. Athymic *Foxn1*^{nu} mice experimentally infected with MAdV-1 develop a lethal wasting disease (Winters and Brown, 1980). Similarly, *Prkdc*^{scid} mice succumb to experimental infection with MAdV-1 (Pirofski *et al.*, 1991).

Gross lesions in response to natural MAdV infections are not detectable. Occasional lesions observed after experimental infection with MAdV-1 include small surface haemorrhages in the brain and spinal cord of C57BL/6 and DBA/2J mice (Guida *et al.*, 1995),

duodenal haemorrhage in *Foxn1*^{nu} mice (Winters and Brown, 1980), and pale yellow livers in *Prkdc*^{scid} mice (Pirofski *et al.*, 1991).

Histologically, experimental MAdV-1 infection of suckling mice is characterized by multifocal necrosis and large basophilic intranuclear inclusion bodies in liver, adrenal gland, heart, kidney, salivary glands, spleen, brain, pancreas, and brown fat (Heck *et al.*, 1972; Margolis *et al.*, 1974; National Research Council, 1991; Percy and Barthold, 2001). In experimentally induced haemorrhagic encephalomyelitis, multifocal petechial haemorrhages occur throughout the brain and spinal cord, predominantly in the white matter, and are attributed to infection and damage to the vascular epithelium of the central nervous system (CNS; Guida *et al.*, 1995). Histopathological manifestations in MAdV-1-infected *Prkdc*^{scid} mice are marked by microvesicular fatty degeneration of hepatocytes (Pirofski *et al.*, 1991). In contrast to MAdV-1, the tissue tropism of MAdV-2 is limited to the intestinal epithelium. Naturally or experimentally infected mice develop intranuclear inclusions in enterocytes, especially in the ileum and caecum (Takeuchi and Hashimoto, 1976; Otten and Tennant, 1982; National Research Council, 1991; Percy and Barthold, 2001).

Transmission of MAdV primarily occurs by ingestion. MAdV-1 is excreted in the urine and may be shed for up to 2 years (Van der Veen and Mes, 1973). Murine adenovirus-2 infects the intestinal tract and is shed in faeces for only a few weeks in immunocompetent mice (Hashimoto *et al.*, 1970); immunodeficient mice may shed the virus for longer periods (Umehara *et al.*, 1984).

Murine adenovirus infections are routinely diagnosed by serological tests. However, there is a one-sided cross reactivity of MAdV-1 with MAdV-2 (Wigand *et al.*, 1977). Serum from mice experimentally infected with MAdV-1 yielded positive reactions in serological tests with both viruses while serum from mice infected with MAdV-2 reacted only with the homologous antigen (Lussier *et al.*, 1987). Smith *et al.* (1986) reported that sera may react with MAdV-1 or MAdV-2 or both antigens. Occasional reports of mice with lesions suggestive of adenovirus infections and negative serology (with MAdV-1) indicate that the infection may not be detected if only one virus is used as antigen (Luethans and Wagner, 1983). It has therefore become standard practice to test sera for antibodies to both MAdV-1 and MAdV-2. The common methods are IFA and ELISA, and both are more sensitive than the previously used complement fixation test.

The low prevalence in colonies of laboratory mice indicate that MAdV can easily be eliminated (e.g. by hysterectomy derivation or embryo transfer) and that barrier maintenance has been very effective in preventing infection.

The low pathogenicity and the low prevalence in contemporary mouse populations are the main reasons why adenoviruses are considered to be of little importance. However, immunodeficient mice are increasingly used and candidates for natural infections and wasting disease (Richter, 1986), and the viruses might easily be spread by the exchange of genetically modified mice and therefore re-emerge. Only few influences on research attributable to MAdV have been published. For example, it has been shown that MAdV-1 significantly aggravates the clinical course of scrapie disease in mice (Ehresmann and Hogan, 1986). Natural infections with MAdV could also interfere with studies using adenovirus as a gene vector.

Polyomaviruses

Polyomaviridae are enveloped, double-stranded DNA viruses. Two different agents of this family exclusively infect mice (*Mus musculus*), and both belong to the genus Polyomavirus. Murine pneumotropic virus (MPtV) has formerly been known as 'newborn mouse pneumonitis virus' or 'K virus' (named after L. Kilham who first described the virus). The second is murine polyomavirus (MPyV). Both are related but antigenically distinct from each other (Bond *et al.*, 1978). They are **enzootic** in many populations of wild mice but are very uncommon in laboratory mice. Even older reports indicate that both have been eradicated from the vast majority of contemporary mouse colonies, and their importance is negligible (National Research Council, 1991). Seropositivity to these viruses was not reported in a survey conducted in the USA (Jacoby and Lindsey, 1998). In a retrospective study in French facilities, antibodies to MPyV were found in 1 of 69 colonies, and all samples tested for MPtV were negative (Zenner and Regnault, 2000). Comparable data were reported by Livingston and Riley (2003). Due to their low prevalence, both viruses are not included in the list of agents for which testing is recommended on a regular basis by FELASA (Nicklas *et al.*, 2002).

Although polyomavirus genes, especially those of SV40 are used widely in gene constructs for insertional mutagenesis, very few reports have been published on spontaneous or experimental disease due to MPyV or MPtV in the last 10–15 years. The reader is therefore referred to previous review articles for details (Eddy 1982; Parker and Richter, 1982; Richter, 1986; Shah

and Christian, 1986; National Research Council, 1991; Orcutt, 1994; Porterfield and Richter, 1994).

MPtV

Natural infections with MPtV are subclinical. The prevalence of infection is usually low in an infected population. The virus may persist in infected animals for months and perhaps for life depending on the age at infection and is reactivated under conditions of immunosuppression. Virus replicates primarily in endothelial cells, but renal tubular epithelial cells are the major site of viral persistence (Greenlee *et al.*, 1991, 1994).

Clinical signs are observed only after infection of infant mice less than 6–8 days of age. Infected pups suddenly develop respiratory symptoms after an incubation period of approximately 1 week, and many die within a few hours of onset of symptoms with an interstitial pneumonia caused by productive infection of and damage to pulmonary endothelium. Endothelial cells in other organs are involved in virus replication also (Ikeda *et al.*, 1988; Greenlee *et al.*, 1994). In older suckling mice, MPtV produces a more protracted infection, and the virus or viral antigen can be detected for as long as 4 months. In adult animals, the virus produces a transient asymptomatic infection. Even in immunodeficient *Foxn1*^{nu} mice, experimental infection of adults is clinically asymptomatic although virus is detectable for a period of several months (Greenlee, 1986).

In vitro cultivation of MPtV is difficult. No susceptible permanent cell line is known to support growth. It can be cultured in primary mouse embryonic cells, but viral titres are not sufficient for use in serological assays (Greenlee and Dodd, 1987). For this reason, the **HI test** using homogenates of livers and lungs of infected newborn mice is still frequently used, but IFA and ELISA tests are also available (Groen *et al.*, 1989). Furthermore, a PCR test for demonstration of MPtV in biological samples has been published (Carty *et al.*, 2001).

MPyV

Murine polyomavirus was first detected as a contaminant of murine leukaemia virus (MuLV) when sarcomas developed in mice after experimental inoculation of contaminated samples. It has later been shown to be a frequent contaminant of transplantable tumours (Collins and Parker, 1972). Natural infection of mice is subclinical, and gross lesions including tumours are usually not found. Tumour formation occurs if mice are experimentally infected at a young age or if they are inoculated with high virus doses. Development of tumours may be preceded by multifocal necrosis and mortality during the viraemic stage (Percy and Barthold, 2001). Parotid, salivary gland, and mammary tumours are common, and sarcomas or carcinomas of kidney, subcutis, adrenal glands, bone, cartilage, teeth, blood vessels, and thyroid occur also. Virus strains vary with regard to the tumour types or lesions that they induce, and mouse strains vary in their susceptibility to different tumour types. Those of C57BL and C57Br/cd lineage are considered to be the most resistant strains; athymic *Foxn1*^{nu} mice are considered to be most susceptible; C3H mice are particularly susceptible to adrenal tumours and A mice tend to develop bone tumours. Immunosuppression or inoculation into immunodeficient strains (e.g. *Foxn1*^{nu}) also support the growth of tumours. On the other hand, experimental infection of adult immunocompetent mice does not result in tumour formation because the immune response suppresses tumour growth, and newborn immunocompetent mice develop runting only if inoculated with high virus doses (Atencio *et al.*, 1995).

After experimental intranasal infection, MPyV initially infects the respiratory tract followed by a systemic phase in which liver, spleen, kidney, and the colon become infected (Dubensky *et al.*, 1984). The virus is shed in faeces and in all body fluids, and transmission occurs rapidly by direct contact between animals, but also between cages in a room. Further, intrauterine transmission has been documented after experimental infection (McCance and Mims, 1977). Murine polyomavirus persists in all organs in *Prkdc*^{scid} mice while viral DNA is detectable in immunocompetent mice after experimental infection for only a limited period of about 4 weeks (Berke *et al.*, 1994). However, virus may persist and can be reactivated by prolonged immunosuppression (Rubino and Walker, 1988) or during pregnancy, at least in young mice (McCance and Mims, 1979). Biological materials of mouse origin are likely to be the most common source of contamination of laboratory mice emphasizing the importance of MAP or PCR screening of biological materials to be inoculated into mice.

The most frequently used tests for health surveillance of mouse colonies are ELISA and IFA (ACLAD, 1991); in addition, the HI test is still used. Latent infections can be detected by intracerebral inoculation of neonate mice or by MAP testing, but direct demonstration of virus in biological samples is also possible by PCR testing (Porterfield and Richter, 1994; Carty *et al.*, 2001).

Parvoviruses

Parvoviruses are non-enveloped small viruses (approximately 20 nm in diameter) with a single-stranded DNA genome of approximately 5000 nucleotides. Murine parvoviruses are members of the family Parvoviridae, genus Parvovirus. They are remarkably resistant to environmental conditions like heat, desiccation, acidic and basic pH-values. Two distinct serotypes infect laboratory mice: the mice minute virus (MMV) and the mouse parvovirus 1 (MPV). Non-structural proteins (NS-1 and NS-2) are highly conserved among both viruses whereas the capsid proteins (VP-1, VP-2, VP-3) are more divergent and determine the serogroup (Ball-Goodrich and Johnson, 1994). Both viruses require mitotically active cells for replication. Severe infections are therefore not found in mature animals due to the lack of a sufficient number of susceptible cells in tissues. General aspects of rodent parvovirus infections and their potential effects on research results have been reviewed (Tattersall and Cotmore, 1986; National Research Council, 1991; Jacoby and Ball-Goodrich, 1995; Jacoby *et al.*, 1996).

MPV

Already in the mid-1980s mouse colonies were identified that gave positive reactions for MMV by IFA but not by HI tests. It was subsequently shown that these colonies were infected with a novel parvovirus, initially referred to as 'mouse orphan parvovirus'. The first isolate of MPV was detected as a contaminant of cultivated T-cell clones interfering with *in vitro* immune responses (McKisic *et al.*, 1993) and was named 'mouse parvovirus'. It does not replicate well in currently available cell cultures, and sufficient quantities of virus for serological tests are difficult to generate. Hitherto, only very few isolates of MPV have been cultured and characterized on a molecular basis (Ball-Goodrich and Johnson, 1994; Besselsen *et al.*, 1996).

At present, MPV is among the most common viruses in colonies of laboratory mice. The prevalence of sera positive for parvoviruses was nearly 10% in a study from Livingston *et al.* (2002), with the majority of sera being positive for MPV. This is consistent with a recent survey conducted in the USA showing that almost 40% of non-SPF colonies were seropositive (Jacoby and Lindsey, 1998). Similar results were obtained for genetically modified mice in Japan (Yamamoto *et al.*, 2001), in contrast to earlier studies indicating that the infection was rare in Japan (Ueno *et al.*, 1998).

Clinical disease and gross or histological lesions have not been reported for mice naturally or experimentally infected with MPV. Infections are subclinical even in newborn and immunocompromised animals (Smith *et al.*, 1993a; Jacoby *et al.*, 1995). In contrast to many other viruses infecting mice, viral replication and excretion is not terminated by the onset of host immunity. Tissue necrosis has not been observed at any stage of infection in infected infant or adult mice (Smith *et al.*, 1993a; Jacoby *et al.*, 1995). Humoral immunity to MPV does not protect against MMV infections and vice versa (Hansen *et al.*, 1999).

Serological surveys have indicated that MPV naturally infects only mice. Differences in mouse strain susceptibility to clinical MPV infection do not exist. However, seroconversion seems to be strain-dependent. After experimental infection, seroconversion occurred in all C3H/HeN mice, fewer BALB/c, DBA/2, and ICR mice, and seroconversion could not be detected in C57BL/6 mice (Besselsen *et al.*, 2000). Diagnosis of MPV infection by PCR testing of small intestine and mesenteric lymph nodes also depended on the mouse strain. MPV DNA was detected in all mouse strains evaluated except DBA/2 even though seroconversion was detected in these mice.

After oral infection, the intestine is the primary site of viral entry and replication. The virus spreads to the mesenteric lymph nodes and other lymphoid tissues, where it persists for more than 2 months (Jacoby *et al.*, 1995), and seems to be excreted via the intestinal and the urinary tract. After experimental inoculation of weanling mice, MPV is transmitted to cagemates by direct contact for 2–4 weeks (Smith *et al.*, 1993a), and transmission by dirty bedding is also possible. These results implicate a role for urinary, faecal, and perhaps respiratory excretion of virus. Another study showed that naturally infected mice may not transmit the virus under similar experimental conditions (Shek *et al.*, 1998).

Serology is a useful tool to identify MPV infections in immunocompetent hosts, but reaching a diagnosis based on serological assays may be difficult and requires a good knowledge of the available techniques. Neither the virion ELISA nor HI are practical screening tests for MPV because they require large quantities of purified MPV which is difficult to obtain. Diagnosis of MPV infections has long been made on the basis of an MMV HI-negative result coupled with an MMV IFA-positive result. A generic rodent parvovirus ELISA using a recombinant NS-1 protein as antigen has been developed (Riley *et al.*, 1996), but MPV IFA and MPV HI assays are more sensitive techniques than the NS-1

ELISA and the MMV IFA (Besselsen *et al.*, 2000). Recently, ELISA tests have been described that use recombinant VP-2 and provide sensitive and serogroup-specific assays for the diagnosis of MPV infections in mice (Ball-Goodrich *et al.*, 2002; Livingston *et al.*, 2002). In immunodeficient mice that do not generate a humoral immune response, PCR assays can be used to detect MPV (Besselsen *et al.*, 1995; Redig and Besselsen, 2001) and other parvoviruses. MPV has been shown to persist for at least 9 weeks in the mesenteric lymph nodes (Jacoby *et al.*, 1995). This tissue is considered the best suited for PCR analysis, but spleen and small intestine can also be used with good success (Besselsen *et al.*, 2000). The virus persists sufficiently long in mesenteric lymph nodes so that PCR assays may also be used as a primary screening tool for laboratories that do not have access to specific MPV antigen-based serological assays. Polymerase chain reaction is further a good confirmatory method for serological assays and has also been described for the detection of parvoviruses in cell lines and tumours (Yagami *et al.*, 1995). In addition, the MAP test has been reported as a sensitive tool to detect MPV (Shek *et al.*, 1998).

Given the high environmental stability of the virus and the potential **fomite** transmission together with the long virus persistence in infected animals, spontaneous disappearance from a mouse population (e.g. by cessation of breeding) is very unlikely. Eradication of infection is possible by elimination of infected animals and subsequent replacement with uninfected mice, and the agent can be eliminated from breeding populations only by embryo transfer or by hysterectomy.

Although there are few published reports of confounding effects of MPV on research, it is lymphocytotropic and may perturb immune responses *in vitro* and *in vivo*. Infections with MPV have been shown to influence rejection of skin and tumour grafts (McKisic *et al.*, 1996, 1998).

MMV

Mice minute virus is the type species of the genus Parvovirus. The virus was formerly called 'minute virus of mice' (MVM) and was renamed recently (International Union of Microbiological Societies, 2000). It was originally isolated by Crawford (1966) from a stock of mouse adenovirus, and this prototype isolate was later designated MVMp. Its allotropic variant was detected as a contaminant of a transplantable mouse lymphoma (Bonnard *et al.*, 1976) and designated MVMi because it exhibits immunosuppressive properties *in vitro*. Both variants have distinct cell tropisms *in vivo* and *in vitro*. The MMVp infects fibroblast cell lines and does not cause clinical disease (Kimsey *et al.*, 1986, Brownstein *et al.*, 1991). The MMVi grows lytically in T cells and inhibits various functions mediated by these cells *in vitro*. Both strains are apathogenic for adult mice, but the immunosuppressive variant is more pathogenic for neonatal mice than is MMVp.

Serological surveys show that the mouse is the primary natural host (Parker *et al.*, 1970; Smith *et al.*, 1993b; Singleton *et al.*, 2000), but the virus is also infective for rats, hamsters (Garant *et al.*, 1980; Ward and Tattersall, 1982), and *Mastomys* (Haag *et al.*, 2000) during foetal development or after parenteral inoculation.

Natural infections are usually asymptomatic in adults and infants, and the most common sign of infection is seroconversion. Kilham and Margolis (1970) observed mild growth retardation a few days after experimental infection of neonatal mice with MMVp. Studies of transplacental infection yielded no pathological findings in mice (Kilham and Margolis, 1971). The immunosuppressive variant but not the prototype strain is able to produce a runting syndrome after experimental infection of newborn mice (Kimsey *et al.*, 1986). Depending on the host genotype, experimental infections of foetal and neonatal mice with MMVi produce various clinical presentations and lesions. Infection in C57BL/6 mice is asymptomatic, but the virus causes lethal infections with intestinal haemorrhage in DBA/2 mice. Infection of strains such as BALB/c, CBA, C3H/He, and SJL is also lethal and mice have renal papillary haemorrhage (Brownstein *et al.*, 1991). The MMVi also infects haematopoietic stem cells and mediates an acute **myelosuppression** (Segovia *et al.*, 1991, 1995). Due to their dependency on mitotically active tissues, the foetus is at particular risk for damage by parvoviruses. Mice minute virus and other parvoviruses may have severe teratogenic effects and cause foetal and neonatal abnormalities by destroying rapidly dividing cell populations, often resulting in foetal death. Adult *Prkdc*^{scid} mice develop an acute leukopenia 1 month after experimental infection with MMVi and die within 3 months. The virus persists lifelong in the bone marrow of these mice (Segovia *et al.*, 1999).

Mice minute virus is shed in faeces and urine. Contaminated food and bedding are important factors in viral transmission because the virus is very resistant to environmental conditions. Direct contact is also important and the virus does not easily spread between cages.

Routine health surveillance is usually conducted by serological methods. Unlike MPV, MMV can easily be

cultured in cell lines so that antigen production for HI and ELISA (using whole purified virions) is easy. Haemagglutination inhibition is a highly specific diagnostic test whereas IFA always exhibits some degree of cross reactivity with MPV and other closely related parvoviruses. Enzyme-linked immunosorbent assay is probably the most frequently used test, but depending on the purity of the antigen preparation, cross reactions with MPV may occur due to contamination with non-structural proteins that are common to both viruses. This problem can be avoided by the use of recombinant VP-2 antigen (Livingston *et al.*, 2002). Viral detection is also possible by PCR in biological materials and in organs (intestines, kidney, spleen) from infected animals (Yagami *et al.*, 1995; Chang *et al.*, 1997; Redig and Besselsen, 2001). In contrast to MPV, PCR is not appropriate as a confirmatory method for serology because MMV has not been shown to persist in immunocompetent animals for sufficiently long periods.

The virus can be eliminated from infected breeding populations by caesarean derivation or by embryo transfer. In experimental colonies, elimination of infected animals and subsequent replacement with uninfected mice is practical if careful environmental sanitation is conducted by appropriate disinfection procedures. It is important that reintroduction is avoided by exclusion of wild mice and by strict separation from other infected populations and potentially contaminated materials in the same facility. Admission of biological materials must be restricted to samples that have been tested and found free from viral contamination.

Both allotropic variants of MMV have been used as models for molecular virology, and their small size and simple structure have facilitated examination of their molecular biology and expedited understanding of cell tropism, viral genetics, and structure. The significance for laboratory mouse populations was considered low or uncertain because natural infections are inapparent. However, various effects on mouse-based research have been published (Tattersall and Cotmore, 1986; Jacoby *et al.*, 1996; Baker, 1998; Nicklas *et al.*, 1999). Due to their predilection for replicating in mitotically active cells, they are frequently associated with tumour cells and have a marked **oncosuppressive** effect (Rommelaere and Cornelis, 1991). Special attention is also necessary for immunological research and other studies involving rapidly dividing cells (embryology, teratology). In addition, MMV is a common contaminant of transplantable tumours, murine leukaemias, and other cell lines (Collins and Parker, 1972; Nicklas *et al.*, 1993; Garnick, 1996).

RNA viruses

Lactate dehydrogenase-elevating virus

Lactate dehydrogenase-elevating virus (LDV) is a single-stranded RNA virus of the genus Arterivirus belonging to the family Arteriviridae. Lactate dehydrogenase-elevating virus has repeatedly been detected in feral mice (*Mus musculus*), which are considered to be a virus reservoir (Rowson and Mahy, 1975; Li *et al.*, 2000). Only mice and primary mouse cells are susceptible to infection with LDV. After infection, virus titres of 10^{10}–10^{11} particles per ml serum are found within 12–14 h after infection. The virus titre drops to 10^5 particles per ml within 2–3 weeks and remains constant at this level for life. Lactate dehydrogenase-elevating virus replicates in a subpopulation of macrophages in almost all tissues and persists in lymph nodes, spleen, liver, and testes tissues (Anderson *et al.*, 1995a). The virus can be stored in undiluted mouse plasma at −70°C without loss of infectivity, but it is not stable at room temperature and is very sensitive to environmental conditions.

Lactate dehydrogenase-elevating virus was first detected during a study of methods that could be used in the early diagnosis of tumours (Riley *et al.*, 1960). It produces a persistent infection with continuous virus production and a lifelong viraemia despite LDV-specific immune reactions of the host (Van den Broek *et al.*, 1997). Lactate dehydrogenase-elevating virus has been found in numerous biological materials that are serially passaged in mice such as transplantable tumours including human tumours (Nicklas *et al.*, 1993; Ohnishi *et al.*, 1995), monoclonal antibodies or ascitic fluids (Nicklas *et al.*, 1988), or infectious agents (e.g. haemoprotozoans, K virus, *Clostridium piliforme*). These materials are contaminated after passage in an infected and viraemic animal. Contamination with LDV leads to the infection of each sequential host and to transmission of the virus by the next passage and remains associated with the specimen. It is therefore the most frequently detected contaminant in biological materials (Collins and Parker, 1972; Nicklas *et al.*, 1993).

Infection with LDV is usually asymptomatic, and there are no gross lesions in immunocompetent as well as in immunodeficient mice. The only exception is polyomyelitis with flaccid paralysis of hind limbs developing in C58 and AKR mice when they are immunosuppressed either naturally with aging or

experimentally (Anderson *et al.*, 1995b; Monteyne *et al.*, 1997). It has been shown that only mice harbouring cells in the CNS that express a specific endogenous MuLV are susceptible to poliomyelitis (Anderson *et al.*, 1995c).

The characteristic feature of LDV infection is the increased activity of lactate dehydrogenase (LDH) and other plasma enzymes (Brinton, 1986; National Research Council, 1991), which is due to the continuous destruction of permissive macrophages that are responsible for the clearance of LDH from the circulation. As a consequence, the activity of plasma LDH begins to rise by only 24 h after infection and peaks 3–4 days after infection at 5–10-fold normal levels, or even be up to 20-fold in SJL/J mice. The enzyme activity declines during the next 2 weeks but remains elevated throughout life.

Antigen–antibody complexes produced during infection circulate in the blood and are deposited in the glomeruli (Brinton, 1986; National Research Council, 1991). In contrast to other persistent virus infections (e.g. lymphocytic choriomeningitis virus LCMV), these complexes do not lead to immune complex disease and produce only a very mild glomerulopathy. The only gross finding associated with LDV infection is mild splenomegaly. Microscopically, necrosis of lymphoid tissues is visible during the first days of infection. In mouse strains that are susceptible to poliomyelitis, LDV induces lesions in the grey matter of the spinal cord and the brain stem (Brinton, 1986).

Lactate dehydrogenase-elevating virus is not easily transmitted between mice, even in animals housed in the same cage. Fighting and cannibalism increase transmission between cage mates most likely via blood and saliva. Infected females transmit the virus to their foetuses if they have been infected few days prior to birth and before IgG anti-LDV antibodies are produced, but developmental and immunological factors (e.g. gestational age, timing of maternal infection with LDV, placental barrier) are important in the regulation of transplacental LDV infection (Haven *et al.*, 1996; Zitterkopf *et al.*, 2002). Maternal immunity protects foetuses from intrauterine infection. Immunodeficient *Prkdc*^{scid} mice transmit virus to their offspring also during chronic infection (Broen *et al.*, 1992). An important means of transmission is provided by experimental procedures such as mouse-to-mouse passage of contaminated biological materials or the use of the same needle for sequential inoculation of multiple mice.

In principal, serological methods such as IFA may be used for detecting LDV infection (Hayashi *et al.*, 1992) but they are not of practical importance. Circulating virus–antibody complexes interfere with serological tests (ACLAD, 1991), and sufficient quantities of virus for serological tests are difficult to generate because LDV replicates only in specific subpopulations of primary cultures of murine macrophages and monocytes for one cell cycle (Brinton, 1986). Therefore, diagnosis of LDV infection is primarily based on increased LDH activity in serum or plasma of mice. Lactate dehydrogenase-elevating virus activity in serum or plasma can be measured directly, or samples (e.g. plasma, cell or organ homogenates) are inoculated into pathogen-free mice and the increase in LDH activity within 3–4 days is measured. An 8–10-fold increase is indicative of LDV infection. Detection of infectivity of a plasma sample by the induction of increased LDH activity in the recipient animal is the most reliable means of identifying an infected animal. However, it is important to use clear nonhaemolysed samples because haemolysis will (falsely) elevate activities of multiple serum or plasma enzymes, including LDH. While this assay may be included in a commercial 'MAP test', it does not involve antibody detection. Persistent infection makes LDV an ideal candidate for PCR detection in plasma or in organ homogenates (van der Logt *et al.*, 1994; Chen and Plagemann, 1997). However, reports exist that PCR may produce false-negative results and should be used cautiously (Lipman *et al.*, 2000a). Similarly important as detecting LDV in animals is its detection in biological materials. This may be done by assay for increased LDH activity after inoculation of suspect material into pathogen-free mice (Collins and Parker, 1972; Nicklas *et al.*, 1993) or by PCR (Goto *et al.*, 1998; Bootz and Sieber, 2002).

Lactate dehydrogenase-elevating virus spreads slowly in a population because direct contact is necessary. Therefore LDV-negative breeding populations can easily be established by selecting animals with normal plasma LDH activity. Embryo transfer and hysterectomy derivation are also efficient. The presence of LDV in experimental populations is indicative of contaminated biological materials. In such cases, it is essential that the virus is also eliminated from these samples. This is easily achieved by maintenance of cells by *in vitro* culture instead of by animal-to-animal passages (Plagemann and Swim, 1966). Due to the extreme host specificity of the virus, contaminated tumour samples can also be sanitized by passages in nude rats or other animal species.

Lactate dehydrogenase-elevating virus is a potential confounder of any research using biological materials that are passaged in mice. Once present in an animal, the virus persists lifelong. The most obvious signs are

increased levels of plasma LDH and several other enzymes. Lactate dehydrogenase-elevating virus may also exhibit numerous effects on the immune system (thymus involution, depression of cellular immunity, enhanced or diminished humoral responses, NK cell activation, development of autoimmunity, and suppression of development of diabetes in NOD mice; Cafruny and Hovinen, 1988; Nicklas *et al.*, 1988; Takei *et al.*, 1992; Markine-Goriaynoff *et al.*, 2002; Gomez *et al.*, 2003) and enhance or suppress tumour growth (Brinton, 1982; Baker, 1998; Nicklas *et al.*, 1999).

LCMV

Lymphocytic choriomeningitis virus is an enveloped, segmented single-stranded RNA virus of the genus Arenavirus, family Arenaviridae. Its name refers to the condition that results from experimental intracerebral inoculation of the virus into adult mice and is not considered to be a feature of natural infections. Mice (*Mus musculus*) serve as the natural virus reservoir (Salazar-Bravo *et al.*, 2002), but Syrian hamsters are also important hosts (Ackermann, 1977). Additional species such as rabbits, guinea pigs, squirrels, monkeys, and humans are susceptible to natural or experimental infection. Infection in hamsters is considered to be asymptomatic (National Research Council, 1991). Natural infection of callitrichid primates (marmosets and tamarins) leads to a progressive hepatic disease that is known as 'callitrichid hepatitis' (Montali *et al.*, 1995; Asper *et al.*, 2001; Lukashevich *et al.*, 2003). Antibodies to LCMV have been found in wild mice in Europe (Ackermann *et al.*, 1964), Africa (El Karamany and Imam, 1991), Asia (Morita *et al.*, 1991, 1996), Australia (Smith *et al.*, 1993b), and America (Childs *et al.*, 1992). Thus, it is the only arenavirus with worldwide distribution. Infection with LCMV is rarely found in laboratory mice (Smith *et al.*, 1984). Seropositivity to LCMV was reported in approximately 5% of non-SPF mouse colonies in the USA in 1996 (Jacoby and Lindsey, 1998) and in 4% of French colonies in 1996–97 (Zenner and Regnault, 2000). Recent studies confirm that only a small percentage of mice tested are positive for LCMV (Livingston and Riley, 2003). In addition to laboratory mice and other vertebrate hosts, the virus has frequently been found in transplantable tumours and tissue culture cell lines from mice and hamsters (Bhatt *et al.*, 1986a; Nicklas *et al.*, 1993).

Despite the low prevalence in laboratory mice, seropositivity to this **zoonotic** agent should raise serious concern for human health. Lymphocytic choriomeningitis virus is frequently transmitted to humans from wild mice (Childs *et al.*, 1991) and is also endemic to a varying degree in the human population (Childs *et al.*, 1991; Marrie and Saron, 1998; Lledo *et al.*, 2003) due to contact with wild mice. Lymphocytic choriomeningitis virus is further transmitted to humans by domestic Syrian hamsters (Bowen *et al.*, 1975; Rousseau *et al.*, 1997). In addition, infected laboratory mice (Dykewicz *et al.*, 1992) and contaminated biological materials are important sources of infections for humans, and several outbreaks of LCM among laboratory personnel have been traced to transplantable tumours (Hinman *et al.*, 1975; Biggar *et al.*, 1977; Mahy *et al.*, 1991).

In mice, clinical signs of LCMV infection vary with strain and age of mouse, strain and dose of virus, and route of inoculation (Lehmann-Grube, 1982; National Research Council, 1991). Two forms of natural LCMV infection are generally recognized: a persistent tolerant and an (acute) nontolerant form. The persistent form results from infection of mice that are immunotolerant. This is the case if mice are infected *in utero* or during the first days after birth. This form is characterized by lifelong viraemia and shedding. Mice may show growth retardation, especially during the first 3–4 weeks, but appear otherwise normal. Infectious virus is bound to specific antibodies and complement, and these complexes accumulate in the renal glomeruli, the choroid plexus, and sometimes also in synovial membranes and blood vessel walls. At 7–10 months of age, immune complex nephritis develops with ruffled fur, hunched posture, ascites, and occasional deaths. This immunopathological phenomenon is called 'late onset disease' or 'chronic immune complex disease'. The incidence of this type of disease varies between mouse strains. Gross lesions include enlarged spleen and lymph nodes due to lymphoid hyperplasia. Kidneys affected with glomerulonephritis may be enlarged with a granular surface texture or may be shrunken in later stages of the disease process. Microscopically, there is generalized lymphoid hyperplasia and immune complex deposition in glomeruli and vessel walls, resulting in glomerulonephritis and plasmacytic, lymphocytic perivascular cuffs in all visceral organs (Percy and Barthold, 2001).

The nontolerant acute form occurs when infection is acquired after the development of immunocompetence (in mice older than 1 week). These animals become viraemic but do not shed virus and may die within a few days or weeks. Natural infections of adults are usually asymptomatic. Surviving mice are seropositive and in most cases clear the virus to below detection levels of conventional methods. However, virus may persist at low levels in tissues (particularly spleen, lung, and kidney) of

mice for at least 12 weeks after infection as determined by sensitive assays such as nested reverse transcriptase–polymerase chain reaction (RT-PCR) or immunohistochemisty (Ciurea *et al.*, 1999). Such nonlethal infection leads to protection against otherwise lethal intracerebral challenge. Protection from lethal challenge is also achieved by maternally derived anti-LCMV antibodies through nursing or by the administration of anti-LDV monoclonal IgG2a antibodies (Baldridge and Buchmeier, 1992).

In experimentally infected animals, the route of inoculation (subcutaneous, intraperitoneal, intravenous, intracerebral) also influences the type and degree of disease (Lehmann-Grube, 1982; National Research Council, 1991). Intracerebral inoculation of adult immunocompetent mice typically results in tremors, convulsions, and death due to **meningoencephalitis** and hepatitis. Neurological signs usually appear on day 6 postinoculation, and animals die within 1–3 days after the onset of symptoms or recover within several days. The classic histological picture is of dense perivascular accumulations of lymphocytes and plasma cells in meninges and choroid plexus. While infection following subcutaneous inoculation usually remains inapparent, reaction of mice to intraperitoneal or intravenous inoculation depends on the virus strain and on the mouse strain. Infection by these routes primarily causes multifocal hepatic necrosis and necrosis of lymphoid cells. Athymic *Foxn1*^{nu} mice and other immunodeficient mice do not develop disease but become persistently viraemic and shed virus.

As a general rule, all pathological alterations following LCMV infection are immune-mediated; and mice can be protected from LCMV-induced disease by immunosuppression (Gossmann *et al.*, 1995). Lymphocytic choriomeningitis virus disease is a prototype for virus-induced T-lymphocyte-mediated immune injury and for immune complex disease. For detailed information on the pathogenesis of LCMV infection, the reader is referred to a recent review article by Oldstone (2002). Extensive information on the clinical and pathological features of LCMV infection in mice has been assembled by Lehmann-Grube (1982).

In nature, carrier mice with persistent infection serve as the principal source of virus. Intrauterine transmission is very efficient, and with few exceptions all pups born from carrier mice are infected. Furthermore, persistently infected mice and hamsters can shed large numbers of infectious virions primarily in urine, but also in saliva and milk. The virus can replicate in the gastric mucosa after intragastric infection (Rai *et al.*, 1996, 1997). Gastric inoculation elicits antibody responses of comparable magnitudes as intravenous inoculation and leads to active infection with LCMV indicating that oral infection is possible, e.g., by ingestion of contaminated food or cannibalism. A self-limiting infection frequently results from infection of adult mice. The virus does not spread rapidly after introduction in populations of adult mice, and the infectious chain usually ends. However, if the virus infects a pregnant dam or a newborn mouse, a lifelong infection results, and soon a whole breeding colony of mice may become infected if the mice live in close proximity (which is the case under laboratory conditions).

Lymphocytic choriomeningitis virus is most commonly diagnosed by serological methods. Methods of choice are IFA and ELISA, which have replaced the relatively insensitive complement fixation test. It is important that bleeding of mice is done carefully because of a potential risk due to viraemic animals. Historically, direct viral detection was performed by inoculating body fluids or tissue homogenates into the brain of LCMV-free mice or by subcutaneous injection into mice and subsequent serological testing (MAP test). More recently, PCR assays have been developed for the direct detection of viral RNA in clinical samples or animals (Park *et al.*, 1997, Besselsen *et al.*, 2003). Both MAP test and PCR can also be used to detect contamination of biological materials (Bootz and Sieber, 2002).

Vertical transmission of LCMV by transuterine infection is efficient so this virus cannot reliably be eliminated by caesarean rederivation. Caesarean derivation may be effective if dams acquired infection after the development of **immunocompetence** (nontolerant acute infection) and subsequently eliminated the virus, but such a strategy is difficult to justify in light of LCMV's zoonotic potential. In breeding colonies of great value, virus elimination might be possible soon after introduction into the colony by selecting nonviraemic breeders. This procedure is expensive and time consuming and requires special safety precautions.

Fortunately, infections of laboratory mice with LCMV are very uncommon. However, once LCMV has been detected in animals or in biological materials, immediate destruction of all contaminated animals and materials is advisable to avoid risk of human infection. *Foxn1*^{nu} and *Prkdc*^{scid} mice may pose a special risk because infections are silent and chronic (Mahy *et al.*, 1991). Cages and equipment should be autoclaved, and animal rooms should be fumigated with disinfectants such as formaldehyde, vaporized paraformaldehyde, and hydrogen peroxide.

Appropriate precautions are necessary for experiments involving LCMV, or LCMV-infected animals or

materials. Biological safety level (BSL) 2 will be considered to be sufficient in most cases. Biological safety level 3 practices may be considered when working with infected animals owing to the increased risk of virus transmission by bite wounds, scratching, or aerosol formation from the bedding. Animal Biosafety Level (ABSL) 3 practices and facilities are generally recommended for work with infected hamsters. Appropriate precautions have been defined for different BSLs or animal biology safety levels by CDC (1999).

Lymphocytic choriomeningitis virus is an important zoonotic agent. It has been transmitted to humans working with infected animals or with contaminated biological materials and can cause mild to serious or fatal disease in humans (Dykewicz *et al.*, 1992; Barton *et al.*, 1995; Barton and Hyndman, 2000). Congenital infection in humans may result in hydrocephalus, or foetal or neonatal death (Barton *et al.*, 2002). Lymphocytic choriomeningitis virus is also frequently utilized as a model organism to study virus–host interactions, immunological tolerance, virus-induced immune complex disease, and a number of immunological mechanisms *in vivo* and *in vitro* (Slifka, 2002; Zinkernagel, 2002). Accidental transmission may have a severe impact on various kinds of experiments (for details, see Lehmann-Grube, 1982; Bhatt *et al.*, 1986b; National Research Council, 1991; Baker, 1998; Nicklas *et al.*, 1999).

Mammalian orthoreovirus serotype 3

Mammalian orthoreoviruses (MRV) are nonenveloped, segmented double-stranded RNA viruses of the family Reoviridae, genus Orthoreovirus. They have a wide host range and are ubiquitous throughout the world. The designation *reo* stands for *r*espiratory *e*nteric *o*rphan and reflects the original isolation of these viruses from human respiratory and intestinal tract without apparent disease. The term **'orphan' virus** refers to a virus in search of a disease. Mammalian orthoreovirus can be grouped into three serotypes (1, 2, 3). Mammalian orthoreovirus-3 (synonyms: hepatoencephalomyelitis virus; ECHO 10 virus) infection remains prevalent in contemporary mouse colonies and has been reported in wild mice (Smith *et al.*, 1993b; Barthold, 1997a). Seropositivity to MRV-3 was found in less than 5% of SPF colonies and in approximately 20% of non-SPF mouse colonies in the USA in 1996 (Jacoby and Lindsey, 1998). A study in France reported antibodies to MRV-3 in 9% of mouse colonies examined (Zenner and Regnault, 2000). More recently, a study in North America found a low rate (0.2%) of mouse sera to be positive for antibodies against this virus (Livingston and Riley, 2003). In addition, contamination of mouse origin tumours and cell lines by MRV-3 has been reported many times (National Research Council, 1991; Nicklas *et al.*, 1993; Barthold, 1997a). Experimentally, MRV-3 infection of infant mice has been used to model human hepatobiliary disease, pancreatitis, diabetes mellitus, and lymphoma (Kraft, 1982; National Research Council, 1991; Fenner *et al.*, 1993).

The literature on MRV-3 infections in mice is dominated by studies on experimentally infected animals. The virus can cause severe **pantropic infection** in infant mice (Kraft, 1982; Tyler and Fields, 1986; Barthold, 1997a). After parenteral inoculation, virus can be recovered from the liver, brain, heart, pancreas, spleen, lymph nodes, and blood vessels. Following oral inoculation, reoviruses gain entry by infecting specialized epithelial cells (M cells) that overlie Peyer's patches. The virus then becomes accessible to leukocytes and spreads to other organs by way of the lymphatic system and the bloodstream. Neural spread to the CNS has also been well documented (Morrison *et al.*, 1991). The mechanisms of viral pathogenesis and their interactions with the host cell are reviewed in detail by Tyler (2001) and Tyler *et al.* (2001).

Natural infection by MRV-3 in a mouse colony usually is subclinical although diarrhoea or steatorrhoea and oily hair effect in suckling mice may be noted (Kraft, 1982; Tyler and Fields, 1986; National Research Council, 1991; Barthold, 1997a; Percy and Barthold, 2001). The latter term has been used to describe the matted, unkempt appearance of the hair coat that results from **steatorrhoea** due to pancreatitis, maldigestion, and **biliary atresia**. In addition, runting (attributed to immune-mediated destruction of cells in the pituitary gland that produce growth hormone), transient alopecia, jaundice (due to excessive bilirubin in the blood, which is attributed to the liver pathology, especially biliary atresia), and neurological signs such as incoordination, tremors, or paralysis may develop. When present in natural infections, clinical signs and lesions are similar to but milder than in experimental neonatal infections. Early descriptions of naturally occurring disease may have been complicated by concurrent infections such as MHV or murine rotavirus A (MuRV-A)/epizootic diarrhoea of infant mice (EDIM) virus that contributed to the severity of the lesions especially in liver, pancreas, CNS, and intestine. The outcome of MRV-3 infection depends on age and immunological status of mouse, dose of virus, and

route of inoculation. Adult immunocompetent mice typically show no clinical signs and have no discernible lesions even in experimental infections. Mucosal and maternally conferred immunity are considered to be important in protection from or resolution of disease (Cuff *et al.*, 1990; Barthold *et al.*, 1993b). Experimental infection of adult *Prkdc*^{scid} mice is lethal (George *et al.*, 1990). Depending on the route of inoculation, experimental infection of adult *Foxn1*^{nu} mice is subclinical or results in liver disease (Carthew, 1984; George *et al.*, 1990).

Histological findings reported to occur after experimental MRV-3 infection of neonatal mice include inflammation and necrosis in liver, pancreas, heart, adrenal, brain, and spinal cord; lymphoid depletion in thymus, spleen, and lymph nodes; and hepatic fibrosis with biliary atresia (Papadimitriou and Robertson, 1976; Tyler and Fields, 1986; Barthold *et al.*, 1993b; Barthold, 1997a; Percy and Barthold, 2001).

Transmission of reoviruses probably involves the aerosol as well as the faecal–oral route (National Research Council, 1991). Fomites may play an important role as passive vectors because reoviruses resist environmental conditions moderately well.

Serological screening with ELISA or IFA is in widespread use for detection of antibodies to MRV-3 in diagnostic and health surveillance programmes. Both ELISA and IFA detect cross-reacting antibodies to heterologous MRV serotypes that can infect mice (ACLAD, 1991). The HI test does not detect such cross-reacting antibodies but is prone to give false positive results due to nonspecific inhibitors of haemagglutination (Kraft and Meyer, 1986; Van Der Logt, 1986; ACLAD, 1991). Reverse transcriptase–polymerase chain reaction methods for the detection of MRV-3 RNA (Steele *et al.*, 1995) or MRV RNA (Leary *et al.*, 2002) are also available. Reports on contamination of mouse origin tumours and cell lines by MRV-3 and its interference with transplantable tumour studies (Bennette, 1960; Nelson and Tarnowski, 1960) emphasize the importance of screening of biological materials to be inoculated into mice by MAP test or PCR. Natural seroconversion to MRV-3 without clinical disease is also observed in laboratory rats, hamsters, and guinea pigs (National Research Council, 1991; Barthold, 1997a).

Caesarean derivation and barrier maintenance have proven effective in the control and prevention of MRV-3 infection (Kraft, 1982; National Research Council, 1991).

The virus may interfere with research involving transplantable tumours and cell lines of mouse origin. It has the potential to alter intestinal studies and multiple immune response functions in mice. In enzootically infected colonies, protection of neonates by maternal antibody could complicate or prevent experimental infections with reoviruses. It could further complicate experiments that require evaluation of liver, pancreas, CNS, heart, lymphoid organs, and other tissues affected by the virus.

Murine hepatitis virus

The term murine hepatitis virus (MHV; commonly referred to as 'mouse hepatitis virus') designates a large group of antigenically and genetically related, single-stranded RNA viruses belonging to the family Coronaviridae, genus Coronavirus. They are surrounded by an envelope with a corona of surface projections (spikes). Murine hepatitis virus is antigenically related to rat coronaviruses and other coronaviruses of pigs, cattle, and humans. Numerous different strains or isolates of MHV have been described. They can be distinguished by neutralization tests that detect strain-specific **spike (S) antigens**. The best studied strains are the prototype strains MHV-1, MHV-2, MHV-3, JHM (MHV-4), A59, and S, of which MHV-3 is regarded as the most virulent. Murine hepatitis virus, like other coronaviruses, mutates rapidly, and strains readily form recombinants, so that new (sub)strains are constantly evolving. Strains vary in their virulence, organotropism, and cell tropism (Homberger, 1997). Based on their primary organotropism, MHV strains can be grouped into two biotypes: respiratory (or polytropic) and enterotropic. However, intermediate forms (enterotropic strains with tropism to other organs) exist. Murine hepatitis virus is relatively resistant to repeated freezing and thawing, heating (56°C for 30 min), and acid pH but is sensitive to drying and disinfectants, especially those with detergent activity (National Research Council, 1991).

Mus musculus is the natural host of MHV. It can be found in wild and laboratory mice throughout the world and is one of the most common viral pathogens in contemporary mouse colonies. While polytropic strains have historically been considered more common, this situation is thought to have reversed. A survey conducted in the USA in 1996 reported antibodies to MHV in more than 10% of SPF mouse colonies and more than 70% of non-SPF colonies (Jacoby and Lindsey, 1998), though very recent monitoring results for research institutions across North America indicate that the prevalence of MHV has decreased during the past few years (Livingston and Riley, 2003). A retrospective study in France covering the period from 1988 to 1997

reported antibodies to MHV in 67% of mouse colonies examined (Zenner and Regnault, 2000). Suckling rats inoculated experimentally with MHV had transient virus replication in the nasal mucosa and seroconversion but no clinical disease (Taguchi *et al.*, 1979). Similarly, deer mice seroconverted but showed no clinical disease after experimental infection (Silverman *et al.*, 1982). Murine hepatitis virus is also a common contaminant of transplantable tumours (Collins and Parker, 1972; Nicklas *et al.*, 1993) and cell lines (Sabesin, 1972; Yoshikura and Taguchi, 1979).

The pathogenesis and outcome of MHV infections depend on interactions among numerous factors related to the virus (e.g. virulence and organotropism) and the host (e.g. age, genotype, immune status, and microbiological status; Kraft, 1982; Barthold, 1986; National Research Council, 1991; Compton *et al.*, 1993; Homberger, 1997; Percy and Barthold, 2001). Murine hepatitis virus strains appear to possess a **primary tropism** for the upper respiratory or enteric mucosa. Those strains with respiratory tropism initiate infection in the nasal mucosa and then may disseminate via blood and lymphatics to a variety of other organs because of their polytropic nature. Respiratory (polytropic) strains include MHV-1, MHV-2, MHV-3, A59, S, and JHM. Infection of mice with virulent polytropic MHV strains, infection of mice less than 2 weeks of age, infection of genetically susceptible strains of mice, or infection of immunocompromised mice favour virus dissemination. Virus then secondarily replicates in vascular endothelium and parenchymal tissues, causing disease of brain, liver, lymphoid organs, bone marrow, and other sites. Infection of the brain by viraemic dissemination occurs primarily in immunocompromised or neonatal mice. Additionally, infection of adult mouse brain can occur by extension of virus along olfactory neural pathways, even in the absence of dissemination to other organs. In contrast, enterotropic MHV strains (e.g. LIVIM, MHV-D, and MHV-Y) tend to selectively infect intestinal mucosal epithelium, with no or minimal dissemination to other organs such as mesenteric lymph nodes or liver.

All ages and strains are susceptible to active infection, but disease is largely age-related. Infection of neonatal mice results in severe necrotizing enterocolitis with high mortality within 48 h. Mortality and lesion severity diminish rapidly with advancing age at infection. Adult mice develop minimal lesions although replication of equal or higher titres of virus occurs compared with neonates. The age-dependent decrease in severity of enterotropic MHV disease is probably related to the higher mucosal epithelium turnover in older mice, allowing more rapid replacement of damaged mucosa. Another factor that is of considerable importance to the outcome of MHV infections is host genotype. For example, BALB/c mice are highly susceptible to enterotropic MHV disease while SJL mice, at the other end of the spectrum, are highly resistant (Barthold *et al.*, 1993a). Unlike in polytropic MHV infection where resistance is correlated with reduced virus replication in target cells (Barthold and Smith, 1987), enterotropic MHV grows to comparable titres in SJL and BALB/c mice at all ages (Barthold *et al.*, 1993a). Therefore, the resistance of the SJL mouse to disease caused by enterotropic MHV seems to be mediated through an entirely different mechanism than resistance to polytropic MHV. Furthermore, mouse genotypes that are susceptible to disease caused by one MHV strain may be resistant to disease caused by another strain (Barthold, 1986). It is therefore not possible to strictly categorize mouse strains as susceptible or resistant. The genetic factors determining susceptibility versus resistance in MHV infections are as yet poorly understood. Both polytropic and enterotropic MHV infections are self-limiting in immunocompetent mice. Immune-mediated clearance of virus usually begins about a week after infection, and most mice eliminate the virus within 3–4 weeks (Barthold, 1986; Barthold and Smith, 1990; Barthold *et al.*, 1993a). Humoral and cellular immunity appear to participate in host defences to infection, and functional T cells are an absolute requirement (Williamson and Stohlman, 1990; Kyuwa *et al.*, 1996; Lin *et al.*, 1999; Haring and Perlman, 2001). Therefore, immunodeficient mice such as *Foxn1*^{nu} and *Prkdc*^{scid} mice cannot clear the virus (Barthold *et al.*, 1985; Compton *et al.*, 1993). Similarly, some genetically modified strains of mice may have deficits in antiviral responses or other alterations that allow the development of persistent MHV infection (Rehg *et al.*, 2001). Recovered immune mice are resistant to reinfection with the same MHV strain but remain susceptible to repeated infections with different strains of MHV (Barthold and Smith, 1989a,b; Homberger *et al.*, 1992). Similarly, maternal immunity protects suckling mice against homologous MHV strains but not necessarily against other strains (Homberger and Barthold, 1992; Homberger *et al.*, 1992). However, maternal immunity, even to homologous strains, depends on the presence of maternally acquired antibody in the lumen of the intestine (Homberger and Barthold, 1992). Therefore, the susceptibility of young mice to infection significantly increases at weaning.

Most MHV infections are subclinical and follow one of two epidemiological patterns in immunocompetent

mice (National Research Council, 1991; Homberger, 1997). Enzootic (subclinical) infection, commonly seen in breeding colonies, occurs when a population has been in contact with the virus for a longer period (e.g. several weeks). Adults are immune (due to prior infection), sucklings are passively protected, and infection is perpetuated in weanlings. **Epizootic** (clinical) infection occurs when the virus is introduced into a naive population (housed in open cages). The infection rapidly spreads through the entire colony. Clinical signs depend upon the virus and mouse strains and are most evident in infant mice. Typically, they include diarrhoea, poor growth, lassitude, and death. In infections due to virulent enterotropic strains, mortality can reach 100% in infant mice. Some strains may also cause neurological signs such as flaccid paralysis of hind limbs, convulsions, and circling. Adult infections are again usually asymptomatic. As the infection becomes established in the colony, the epizootic pattern is replaced by the enzootic pattern. In immunodeficient (e.g. *Foxn1*^{nu} and *Prkdc*^{scid}) mice, infection with virulent polytropic MHV strains often is rapidly fatal while less virulent strains cause chronic wasting disease (Compton *et al.*, 1993). In contrast, adult immunodeficient mice can tolerate chronic infection by enterotropic MHV, with slow emaciation and diarrhoea, or minimal clinical disease (Barthold *et al.*, 1985; Barthold, 1986). Subclinical MHV infections can be activated by a variety of experimental procedures (e.g. thymectomy, whole body irradiation, treatment with chemotherapeutic agents, halothane anaesthesia) or by co-infections with other pathogens (e.g. *Eperythrozoon coccoides*, K virus; reviewed by Kraft, 1982; National Research Council, 1991).

In most natural infections, gross lesions are not present or are transient and not observed. Gross findings in neonates with clinical signs include dehydration, emaciation, and in contrast to EDIM, an empty stomach (Ishida *et al.*, 1978; Barthold *et al.*, 1982; Kraft, 1982). The intestine is distended and filled with watery to mucoid yellowish, sometimes gaseous contents. Haemorrhage or rupture of the intestine can occur. Depending on the virus strain, necrotic foci on the liver (Ishida *et al.*, 1978; Kraft, 1982; Percy and Barthold, 2001) and thymus involution (Barthold *et al.*, 1982; Godfraind *et al.*, 1995) may also be seen in susceptible mice. Liver involvement may be accompanied by jaundice and haemorrhagic peritoneal exudate. Splenomegaly may occur as a result of compensatory haematopoiesis (Fox *et al.*, 1977).

Histopathological changes in susceptible mice infected with polytropic MHV strains include acute necrosis with syncytia in liver, spleen, lymph nodes, gut-associated lymphoid tissue, and bone marrow (Kraft, 1982; Barthold, 1986; National Research Council, 1991; Percy and Barthold, 2001). Neonatally infected mice can have vascular-oriented necrotizing (meningo)encephalitis with demyelination in the brain stem and peri-ependymal areas. Lesions in peritoneum, bone marrow, thymus, and other tissues can be variably present. Mice can develop nasoencephalitis due to extension of infection from the nasal mucosa along olfactory pathways to the brain, with meningoencephalitis and demyelination, the latter of which is thought to be largely T cell-mediated (Haring and Perlman, 2001). This pattern of infection regularly occurs after intranasal inoculation of many MHV strains but is a relatively rare event after natural exposure. Syncytia arising from endothelium, parenchyma, or leukocytes is a hallmark of infection in many tissues including intestine, lung, liver, lymph nodes, spleen, thymus, brain, and bone marrow. Lesions are transient and seldom fully developed in adult immunocompetent mice, but they are manifest in immunocompromised mice. Highly unusual presentations can occur in mice with specific gene defects. For example, granulomatous peritonitis and pleuritis were found in interferon-γ-deficient mice infected with MHV (France *et al.*, 1999).

Histopathological changes caused by enterotropic strains of MHV are mainly confined to the intestinal tract and associated lymphoid tissues (Kraft, 1982; Barthold, 1986; National Research Council, 1991; Percy and Barthold, 2001). The most common sites are terminal ileum, caecum, and proximal colon. The severity of disease is primarily age-dependent, with neonatal mice being most severely affected. These mice show segmentally distributed areas of villus attenuation, enterocytic syncytia (balloon cells), and mucosal necrosis accompanied by leukocytic infiltration. Intracytoplasmic inclusions are present in enterocytes. Erosions, ulceration, and haemorrhage may be seen in more severe cases. Lesions can be fully developed within 24–48 h, but are usually more severe at 3–5 days after infection. Surviving mice may develop compensatory mucosal hyperplasia. Mesenteric lymph nodes usually contain lymphocytic syncytia, and mesenteric vessels may contain endothelial syncytia. Pathological changes in older mice are generally much more subtle and may only consist of transient syncytia. An occasional exception seems to occur in immunodeficient animals such as *Foxn1*^{nu} mice, which can develop chronic hyperplastic **typhlocolitis** of varying severity (Barthold *et al.*, 1985), but other agents such as *Helicobacter* species may have been involved. In general, enterotropic

MHV strains do not disseminate, but hepatitis and encephalitis can occur with some virus strains in certain mouse genotypes.

Murine hepatitis virus is highly contagious. It is shed in faeces and nasopharyngeal secretions and appears to be transmitted via direct contact, aerosol, and fomites (Kraft, 1982; National Research Council, 1991). Vertical (*in utero*) transmission has been demonstrated in experimental infections (Katami *et al.*, 1978) but does not seem to be of practical importance under natural conditions.

Diagnosis during the acute stage of infection can be made by histological demonstration of characteristic lesions with syncytia in target tissues, but clinical signs and lesions can be highly variable and may not be prominent. Suckling, genetically susceptible or immunocompromised mice are the best candidates for evaluation. Active infection can be confirmed by immunohistochemistry (Brownstein and Barthold, 1982) or by virus isolation. Virus recovery from infected tissues is difficult but can be accomplished using primary macrophage cultures or a number of established cell lines such as NCTC 1469 or DBT (ACLAD, 1991). These cells, however, may not be successful substrates for some enterotropic MHV strains. Virus in suspect tissue can also be confirmed by bioassays such as MAP testing or infant or *Foxn1*^{nu} mouse inoculation (De Souza and Smith, 1989; ACLAD, 1991). Amplification by passage in these mice increases the likelihood of detection of lesions and antigen, or virus recovery. Other direct diagnostic methods that have been successfully utilized to detect MHV in faeces or tissue of infected mice include monoclonal antibody solution hybridization assay (Casebolt and Stephensen, 1992) and a number of RT-PCR assays (Homberger *et al.*, 1991; Kunita *et al.*, 1992; Yamada *et al.*, 1993; Besselsen *et al.*, 2002). Because of the transient nature of MHV infection in immunocompetent mice, serology is the most appropriate diagnostic tool for routine monitoring. Enzyme-linked immunosorbent assay and IFA are well established and sensitive, and all known MHV strains cross-react in both tests (Smith, 1983; ACLAD, 1991). The magnitude of antibody response depends on MHV strain and mouse genotype (Nakanaga *et al.*, 1983; Barthold and Smith, 1987). DBA/2 mice are poor antibody responders whereas C57BL/6 mice produce a high antibody titre and are therefore good sentinels. Antibody titres remain high over a period of at least 6 months (Barthold and Smith, 1989b; Homberger *et al.*, 1992). Infected mice may not develop detectable antibodies for up to 14 days after initial exposure (Smith, 1983). In such cases, a direct diagnostic method as discussed above may be useful. Another drawback of serology is that mice weaned from immune dams can have maternal antibodies until they are 10 weeks of age (Homberger, 1992). This may impact serological monitoring because the possibility must be considered that low positive results are due to maternally-derived passive immunity. Because the virus can be transmitted by transplantable tumours and other biological materials from mice, including hybridomas (Holmes *et al.*, 1986) and embryonic stem cells (Okumura *et al.*, 1996; Kyuwa, 1997), these materials should also be routinely screened for MHV contamination. Mouse inoculation bioassay, MAP test, and RT-PCR can be used for this purpose.

The best means of MHV control is to prevent its entry into a facility. This can be accomplished by purchase of mice from virus-free sources and maintenance under effective barrier conditions monitored by a well-designed quality assurance programme. Control of wild mouse populations, proper husbandry and sanitation, and strict monitoring of biological materials that may harbour virus are also important measures to prevent infection. If infection occurs, the most effective elimination strategy is to cull the affected colony and obtain clean replacement stock. However, this is not always a feasible option when working with valuable mice (e.g. genetically modified lines, breeding stocks). Caesarean derivation or embryo transfer can be used to produce virus-free offspring, and foster-nursing also has been reported to be effective (Lipman *et al.*, 1987). Quarantine of an affected colony with no breeding and no introduction of new animals for approximately 2 months has been effective in immunocompetent mice (Weir *et al.*, 1987). The infection is likely to be terminated because MHV requires a constant supply of susceptible animals. This method works best when working with small numbers of mice. Large populations favour the development of new MHV strains that may result in repeated infections with slightly different strains (Adami *et al.*, 1995). It may be practical to select a few future breeders from the infected population and quarantine them for approximately 3 weeks (Compton *et al.*, 1993). This can be achieved in isolators, or in individually ventilated cages if proper handling is guaranteed. After this interval, breeding can resume. The 3-week interval should permit recovery from active infection, and the additional 3-week gestation period effectively extends the total quarantine to 6 weeks. It is advisible to select seropositive breeders because the possibility of active infection is lower in such animals. The breeding cessation strategy may not be successful if

immunodeficient mice are used because they are susceptible to chronic infection and viral excretion (Barthold *et al.*, 1985). Genetically engineered mice of unclear, unknown or deficient immune status pose a special challenge because they may develop unusual manifestations of infection or may be unable to clear virus. Rederivation likely is the most cost effective strategy in such situations. Along with the measures described, proper sanitation and disinfection of caging and animal quarters as well as stringent personal sanitation are essential to eliminate infection. Careful testing with sentinel mice should be applied to evaluate the effectiveness of rederivation. If transplantable tumours are contaminated with MHV, virus elimination can be achieved by passage of tumours in athymic *Whn*^{rnu} rats (Rülicke *et al.*, 1991).

Murine hepatitis virus is one of the most important viral pathogens of laboratory mice and has been intensively studied from a number of research perspectives (e.g. as a model organism for studying coronavirus molecular biology or the pathogenesis of viral-induced demyelinating disease). Numerous reports document the effects of natural and experimental infections with MHV on host physiology and research, especially in the fields of immunology and tumour biology (reviewed by Barthold, 1986; National Research Council, 1991; Compton *et al.*, 1993; Homberger, 1997; Baker, 1998; Nicklas *et al.*, 1999).

Murine pneumonia virus

Murine pneumonia virus, commonly referred to as 'pneumonia virus of mice' (PVM), is an enveloped, single-stranded RNA virus of the family Paramyxoviridae, genus Pneumovirus. It is closely related to human respiratory syncytial virus (HRSV). The virus name is officially abbreviated as 'MPV' according to the International Union of Microbiological Societies (2000); however, the former designation 'PVM' will be used in this chapter to avoid confusion with the official abbreviation of mouse parvovirus 1 (MPV). 'Pneumonia virus of mice' infection is relatively common in colonies of mice and rats throughout the world. Seropositivity to PVM was reported in less than 5% of SPF mouse colonies and in approximately 20% of non-SPF mouse colonies in the USA (Jacoby and Lindsey, 1998). A serological survey in France demonstrated antibodies to PVM in 16% of mouse colonies examined (Zenner and Regnault, 2000). In a more recent study in North America, such antibodies were found in only 0.1% of mice monitored (Livingston and Riley, 2003). Antibodies to PVM have also been detected in hamsters, gerbils, cotton rats, guinea pigs, and rabbits (Parker and Richter, 1982; Richter, 1986; National Research Council, 1991). Experimentally, PVM infection of mice is used as a model for HRSV infection (Domachowske *et al.*, 2000).

In immunocompetent mice, natural infection with PVM is transient and usually not associated with clinical disease or pathological findings (Parker and Richter, 1982; National Research Council, 1991; Brownstein, 1996b). However, natural disease and persistent infection may occur in immunodeficient mice (Carthew and Sparrow, 1980; Richter *et al.*, 1988; Weir *et al.*, 1988). In particular, athymic *Foxn1*^{nu} mice seem to be susceptible to PVM infection, which can result in dyspnoea, cyanosis, emaciation, and death due to pneumonia (Richter *et al.*, 1988; Weir *et al.*, 1988). Similar clinical signs have been reported for experimentally infected, immunocompetent mice (Cook *et al.*, 1998).

Necropsy findings in naturally infected *Foxn1*^{nu} mice include **cachexia** and diffuse pulmonary oedema or lobar consolidation (Weir *et al.*, 1988). Pulmonary consolidation (dark red or grey in colour) also has been found after experimental infection of immunocompetent mice (Brownstein, 1996b).

Histologically, natural infection of *Foxn1*^{nu} mice with PVM presents as interstitial pneumonia (Richter *et al.*, 1988; Weir *et al.*, 1988). Experimental intranasal inoculation of immunocompetent mice can result in rhinitis, erosive bronchiolitis, and interstitial pneumonia with prominent early pulmonary eosinophilia and neutrophilia (Brownstein, 1996b; Domachowske *et al.*, 2000). Hydrocephalus may result from intracerebral inoculation of neonatal mice (Lagace-Simard *et al.*, 1980). Susceptibility to infection is influenced by age of mouse, dose of virus, and a variety of local and systemic stressors (Parker and Richter, 1982; National Research Council, 1991).

Pneumonia virus of mice is labile in the environment and rapidly inactivated at room temperature (Parker and Richter, 1982; National Research Council, 1991). The virus is tropic for the respiratory epithelium (Carthew and Sparrow, 1980; Cook *et al.*, 1998), and transmission is exclusively horizontal via the respiratory tract, mainly by direct contact and aerosol (Parker and Richter, 1982; National Research Council, 1991). Therefore, transmissibility in mouse colonies is low, and infections tend to be focal enzootics.

Serology (ELISA, IFA, or HI) is the primary means of testing mouse colonies for exposure to PVM. Immunohistochemistry has been applied to detect viral antigen in lung sections (Carthew and Sparrow, 1980; Weir *et al.*, 1988), however, proper sampling (see Chapter

on Health Monitoring) is critical for establishing the diagnosis due to the focal nature of the infection. An RT-PCR assay to detect viral RNA in respiratory tract tissues has also been reported (Wagner *et al.*, 2003). However, the use of direct methods requires good timing because the virus is present for only up to about 10 days in immunocompetent mice (Brownstein, 1996b).

Embryo transfer or caesarean derivation followed by barrier maintenance can be used to rear mice that are free of PVM. Because active infection is present in the individual immunocompetent mouse for only a short period, strict isolation of a few (preferably seropositive) mice with the temporary cessation of breeding might also be successful in eliminating the virus (Richter, 1986; National Research Council, 1991).

Pneumonia virus of mice could interfere with studies involving the respiratory tract or immunological measurements in mice. In addition, PVM can have devastating effects on research using immunodeficient mice because they are particularly prone to develop fatal disease (Richter *et al.*, 1988; Weir *et al.*, 1988) or become more susceptible to the deleterious effects of other agents such as *Pneumocystis carinii* (Roths *et al.*, 1993).

MuRV-A/EDIM

Murine rotavirus-A/EDIM (commonly referred to as 'mouse rotavirus' or 'epizootic diarrhoea of infant mice virus') is a nonenveloped, segmented double-stranded RNA virus of the family Reoviridae, genus Rotavirus. It is antigenically classified as a group A rotavirus, similar to rotaviruses of many other species that cause neonatal and infantile gastroenteritis (Fenner *et al.*, 1993). Murine rotavirus-A/EDIM infection remains prevalent in contemporary mouse colonies and appears to occur worldwide. Seropositivity to MuRV-A/EDIM was reported in approximately 5% of SPF colonies and in almost 30% of non-SPF mouse colonies in the USA in 1996 (Jacoby and Lindsey, 1998). More recently, Livingston and Riley (2003) found a low rate (1%) of mouse sera to be positive for antibodies against MuRV-A/EDIM. Experimentally, MuRV-A/EDIM infection in mice is used as a model for human rotavirus infection, especially in investigations on the mechanisms of rotavirus immunity and in the development of vaccination strategies (Ward and McNeal, 1999).

Clinical symptoms following MuRV-A/EDIM infection range from inapparent or mild to severe, sometimes fatal, diarrhoea. 'Epizootic diarrhoea of infant mice' describes the clinical syndrome associated with natural or experimental infection by MuRV-A/EDIM during the first 2 weeks of life (Kraft, 1982; Sheridan and Vonderfecht, 1986; National Research Council, 1991; Barthold, 1997b; Percy and Barthold, 2001). Diarrhoea usually begins around 48 h after infection and persists for about 1 week. Affected suckling mice have soft, yellow faeces that wet and stain the perianal region. In severe instances, the mice may be stunted, have dry scaly skin, or are virtually covered with faecal material. Morbidity is very high but mortality is usually low.

Gross lesions in affected mice are confined to the intestinal tract. The caecum and colon may be distended with gas and watery to paste-like contents that are frequently bright yellow. The stomach of diarrheic mice is almost always filled with milk, and this feature has been reported to be a reliable means to differentiate diarrhoea caused by rotavirus from the diarrhoea caused by MHV infection.

Histopathological changes may be subtle even in animals with significant diarrhoea. They are confined to the small intestine and are most prominent at the apices of villi, where rotaviruses infect and replicate within epithelial cells. Hydropic change of villous epithelial cells is the hallmark finding of acute disease. The villi become shortened, and the cells that initially replace the damaged cells are less differentiated, typically cuboidal instead of columnar, and lack a full complement of enzymes for digestion and absorption, resulting in diarrhoea due to maldigestion and malabsorption. Undigested milk in the small intestine promotes bacterial growth and exerts an osmotic effect, exacerbating damage to the villi. Intestinal fluid and electrolyte secretion is further enhanced by activation of the enteric nervous system (Lundgren *et al.*, 2000) and through the effects of a viral enterotoxin called NSP4 (for nonstructural protein 4; Ball *et al.*, 1996). It is hypothesized that NSP4 is released from virus-infected cells and then triggers a signal transduction pathway that alters epithelial cell permeability and chloride secretion.

Susceptibility to EDIM depends on the age of the host and peaks between 4 and 14 days of age (Kraft, 1982; Sheridan and Vonderfecht, 1986; National Research Council, 1991; Barthold, 1997b; Percy and Barthold, 2001). Mice older than about 2 weeks can still be infected with MuRV-A/EDIM, but small numbers of enterocytes become infected, there is little replication of virus, and diarrhoea does not occur. The exact reason for this age-related resistance to disease is unknown. Pups suckling immune dams are protected against EDIM during their period of disease susceptibility

(Rosé *et al.*, 1998). In general, the infection is self-limiting and resolves within days. Successful viral clearance is promoted by an intact immune response (Feng *et al.*, 1997; McNeal *et al.*, 1997; Rosé *et al.*, 1998), and some immunodeficient mice (e.g. *Prkdc*^{scid} and *Rag2*^{tm1Fwa} mice) may shed virus for extended periods or become persistently infected (Riepenhoff-Talty *et al.*, 1987; Franco and Greenberg, 1995). Protection against MuRV-A/EDIM reinfection is primarily mediated by antibodies (Feng *et al.*, 1997; Rosé *et al.*, 1998).

Murine rotavirus-A/EDIM is highly contagious and transmitted by the faecal–oral route (Kraft, 1982; Sheridan and Vonderfecht, 1986; National Research Council, 1991). Dissemination of the virus occurs through direct contact or contaminated fomites and aerosols. MuRV-A/EDIM is stable at −70°C but otherwise tends to be susceptible to extreme environmental conditions, detergents, and disinfectants.

Enzyme-linked immunosorbent assay and IFA are in widespread use for detection of serum antibodies to MuRV-A/EDIM in diagnostic and health surveillance programmes; other assay systems such as those using latex agglutination are also utilized (Ferner *et al.*, 1987). Rotazyme II is a commercially available ELISA for detection of rotavirus antigen in faeces; however, great care must be used in interpreting the results because some feeds have been reported to cause false positive reactions (Jure *et al.*, 1988). Electron microscopy of faeces of diarrheic pups should reveal typical wheel-shaped rotavirus particles, 60–80 nm in diameter. Reverse transcriptase-polymerase chain reaction also can be used to detect rotavirus RNA in faecal samples (Wilde *et al.*, 1990). Good timing is critical for establishing the diagnosis from faeces because virus is shed for only a few days in immunocompetent mice.

Embryo transfer or caesarean derivation followed by barrier maintenance is recommended for rederivation of breeding stocks (Kraft, 1982; National Research Council, 1991). In immunocompetent mice in which infection is effectively cleared, a breeding suspension strategy combined with excellent sanitation, filter tops, and conscientious serological testing of offspring may also be effective.

Murine rotavirus-A/EDIM has the potential to interfere with any research utilizing suckling mice. It may have a significant impact on studies where the intestinal tract of neonatal or infant mice is the target organ. The infection also poses a problem for infectious disease and immune response studies, particularly those involving enteropathogens in infant mice (Newsome and Coney, 1985). In addition, runting could be interpreted erroneously as the effect of genetic manipulation or other experimental manipulation.

Sendai virus

Sendai virus (SeV) is an enveloped, single-stranded RNA virus of the family Paramyxoviridae, genus Respirovirus. It is antigenically related to human parainfluenza virus 1. The virus was named for Sendai, Japan, where it was first isolated from mice. Infections of mice and rats are relatively common and occur worldwide. In addition, there is evidence that hamsters, guinea pigs, and rabbits are susceptible to infection with SeV (Machii *et al.*, 1989; ACLAD, 1991; National Research Council, 1991; Percy and Palmer, 1997); however, some apparently seropositive guinea pigs may in fact be seropositive to other parainfluenza viruses instead of SeV. Seropositivity to SeV was reported to be absent from SPF mouse colonies and to be approximately 20% in non-SPF mouse colonies in the USA (Jacoby and Lindsey, 1998). A study in France reported antibodies to SeV in 17% of mouse colonies examined (Zenner and Regnault, 2000). A low rate of seropositive mice (0.2%) was found in a recent survey in North America (Livingston and Riley, 2003). Furthermore, SeV can contaminate biological materials (Collins and Parker, 1972).

Sendai virus is pneumotropic and the leading cause of viral respiratory disease in mice. The pneumotropism is partially a consequence of the action of respiratory serine proteases such as tryptase Clara, which activate viral infectivity by specific cleavage of the viral fusion glycoprotein (Tashiro *et al.*, 1999). In addition, the apical budding behaviour of SeV may hinder the spread of virus into subepithelial tissues and subsequently to distant organs via the blood.

Two epidemiologic patterns of SeV infection have been recognized, an enzootic (subclinical) and epizootic (clinically apparent) type (Parker and Richter, 1982; National Research Council, 1991; Brownstein, 1996a). Enzootic infections commonly occur in breeding or open colonies, where the constant supply of susceptible animals perpetuates the infection. In breeding colonies, mice are infected shortly after weaning as maternal antibody levels wane. Normally, the infection is subclinical, with virus persisting for approximately 2 weeks, accompanied by seroconversion that persists for a year or longer. Epizootic infections occur upon first introduction of the virus to a colony and either die out (self-cure) after 2–7 months or become enzootic depending on colony conditions. The epizootic form is generally acute, and morbidity is very high resulting in nearly all susceptible animals becoming infected within a short time. Clinical signs vary and include rough hair coat, hunched posture, chattering, respiratory distress,

prolonged gestation, death of neonates and sucklings, and runting in young mice. Breeding colonies may return to normal productivity in 2 months and thereafter maintain the enzootic pattern of infection. Factors such as strain susceptibility, age, husbandry, transport, and copathogens are important in precipitating overt disease. DBA and 129/J strains of mice are very susceptible to SeV pneumonia whereas SJL/J and C57BL/6/J strains and several outbred stocks are relatively resistant. A/J, BALB/c, and SWR/J are among the strains that show intermediate susceptibility. There is no evidence for persistent infection in immunocompetent mice, but persistent or prolonged infection may occur in immunodeficient mice and can result in wasting and death due to progressive pneumonia (Ward *et al.*, 1976; Iwai *et al.*, 1979; Percy *et al.*, 1994). Clearance of a primary SeV infection is mediated by $CD8^+$ and $CD4^+$ T cell mechanisms (Kast *et al.*, 1986; Hou *et al.*, 1992).

Heavier than normal, consolidated, plum-coloured or grey lungs are a characteristic gross finding in severe SeV pneumonia (Parker and Richter, 1982; National Research Council, 1991; Brownstein, 1996a; Percy and Barthold, 2001). Lymphadenopathy and splenomegaly reflect the vigorous immune response to infection.

Histologically, three phases of disease can be recognized in susceptible immunocompetent mice: acute, reparative, and resolution phases (Brownstein, 1996a; Percy and Barthold, 2001). Lesions of the acute phase, which lasts 8–12 days, are primarily attributed to the cell-mediated immune response that destroys infected respiratory epithelial cells and include necrotizing rhinitis, tracheitis, bronch(iol)itis, and alveolitis. Epithelial syncytiae and cytoplasmic inclusion bodies in infected cells may be seen early in this phase. Alveoli contain sloughed necrotic epithelium, fibrin, neutrophils, and mononuclear cells. Atelectasis, bronchiectasis, and emphysema may occur as a result of damage and obstruction of airways. The reparative phase, which may overlap the acute phase but continues through about the third week post infection, is indicated by regeneration of airway lining epithelium. Adenomatous hyperplasia and squamous metaplasia (with multilayered flat epithelial cells instead of normal columnar cells) in the terminal bronchioles and alveoli are considered to be a hallmark of SeV pneumonia. Mixed inflammatory cell infiltrates in this phase tend to be primarily interstitial rather than alveolar as they are in the acute phase. The resolution phase may be complete by the fourth week post infection and lesions may be difficult to identify subsequently. Residual, persistent lesions that may occur include organizing alveolitis and bronchiolitis fibrosa obliterans. Alveoli and bronchioles are replaced by collagen and fibroblasts, foamy macrophages, and lymphoid infiltrates, often with foci of emphysema, cholesterol crystals, and other debris, which represent attempts to organize and wall off residual necrotic debris and fibrin. Lesions are more severe and variable when additional pathogens such as *Mycoplasma pulmonis* are present (National Research Council, 1991). Otitis media has also been reported in natural infections with SeV although some of these studies have been complicated by the presence of other pathogens (Ward, 1974). Sendai virus has been detected in the inner ear after experimental intracerebral inoculation of neonatal mice (Shimokata *et al.*, 1977).

Sendai virus is extremely contagious. Infectious virus is shed during the first 2 weeks of infection and appears to be transmitted by direct contact, contaminated fomites, and respiratory aerosol (Parker and Reynolds, 1968; Parker and Richter, 1982; National Research Council, 1991).

Serology (ELISA, IFA, or HI) is the approach of choice for routine monitoring because serum antibodies to SeV are detectable soon after infection and persist at high levels for many months, although active infection lasts only 1–2 weeks in immunocompetent mice. The short period of active infection limits the utility of direct methods such as immunohistochemistry (Carthew and Sparrow, 1980) and RT-PCR (Hayase *et al.*, 1997; Wagner *et al.*, 2003). Although SeV is considered to be highly contagious, studies have shown that dirty bedding sentinel systems do not reliably detect the infection and that outbred stocks may not seroconvert consistently (Dillehay *et al.*, 1990; Artwohl *et al.*, 1994). Mouse antibody production test and RT-PCR can be used to detect SeV in contaminated biological materials.

Sendai virus infection in mouse colonies has proven to be one of the most difficult virus infections to control because the virus is highly infectious and easily disseminated. Depopulation of infected colonies probably is the most appropriate means to eliminate the virus in most situations. Embryo transfer followed by barrier maintenance has also been used successfully in eliminating the virus (National Research Council, 1991). A less effective alternative is to place the infected animals under strict quarantine, remove all young and pregnant mice, suspend all breeding, and prevent addition of other susceptible animals for approximately 2 months until the infection is extinguished and then breeding and other normal acitivities are resumed (Parker and Richter, 1982; National Research Council,

1991). Vaccines against the virus have been developed (Brownstein, 1986; National Research Council, 1991), but these probably do not represent a practical means to achieve or maintain the seronegative status of colonies that is in demand today.

Sendai virus has the potential to interfere with a wide variety of research involving mice. Reported effects include interference with early embryonic development and foetal growth; alterations of macrophage, NK cell, and T and B cell function; altered responses to transplantable tumours and respiratory carcinogens; altered isograft rejection; and delayed wound healing (reviewed by National Research Council, 1991; Baker, 1998; Nicklas *et al.*, 1999). Pulmonary changes during SeV infection can compromise interpretation of experimentally induced lesions and may lead to opportunistic infections by other agents. They could also affect the response to anaesthetics. In addition, natural SeV infection would interfere with studies using SeV as a gene vector.

Theiler's murine encephalomyelitis virus

Theiler's murine encephalomyelitis virus (TMEV) or murine poliovirus is a member of the genus Cardiovirus in the family Picornaviridae. Members of this genus are nonenveloped viruses with single-stranded RNA. The virus is rapidly destroyed at temperatures above 50°C. It is considered to be a primary pathogen of the CNS of mice and can cause clinical disease resembling that due to poliomyelitis virus infections in humans. Antibodies to TMEV have been identified in mouse colonies and feral populations worldwide, and *Mus musculus* is considered to be the natural host of TMEV (Lipton *et al.*, 2001). The most well-known and most frequently mentioned TMEV strain is GDVII, which is virulent for mice. Infant or young hamsters and laboratory rats are also susceptible to intracerebral infection. The original isolate is designated TO (Theiler's original) and represents a group of TMEV strains with low virulence for mice. Many additional virus strains have been isolated and studied, and they all fall in the broad grouping of TO and GDVII. A similar virus strain has also been isolated from rats, but in contrast to mouse isolates this virus is not pathogenic for rats and mice after intracerebral inoculation (Hemelt *et al.*, 1974). Recently, another rat isolate has been characterized and shown to be most closely related to but quite distinct from other TMEV viruses (Ohsawa *et al.*, 2003). Antibodies to TMEV (strain GDVII) have been detected in guinea pigs and are considered to indicate infection with another closely related cardiovirus (Hansen *et al.*, 1997).

Seropositivity to TMEV was reported in approximately 5% of SPF mouse colonies and approximately 35% of non-SPF mouse colonies in the USA (Jacoby and Lindsey, 1998). Zenner and Regnault (2000) reported a prevalence rate of 9% in French mouse colonies in a retrospective study, and it has been one of the most common virus infections in rodent colonies. In a recent study, antibodies were found in 0.2% of mice monitored (Livingston and Riley, 2003) indicating that TMEV, like most viruses, has meanwhile been eliminated from the majority of mouse colonies.

Theiler's murine encephalomyelitis virus is primarily an enteric pathogen, and virus strains are enterotropic. In natural infections, virus can be detected in intestinal mucosa and faecal matter, and in some cases it is also found in the mesenteric lymph nodes. However, histological lesions in the intestine are not discerned. Virus may be shed via intestinal contents for up to 22 weeks, sometimes intermittently (Brownstein *et al.*, 1989a), and transmission under natural conditions is via the faecal–oral route by direct contact between mice as well as by indirect contact (e.g. dirty bedding). The host immune response limits virus spread, but it does not immediately terminate virus replication in the intestines. Virus is cleared from extraneural tissues, but it persists in the CNS for at least a year.

Clinical disease due to natural TMEV infection is rare, with a rate of only 1 in 1000–10,000 infected immunocompetent animals (Percy and Barthold, 2001). In immunodeficient mice, especially in weanlings, clinical signs may be more common and mortality may be higher (Rozengurt and Sanchez, 1993). This group of viruses usually causes asymptomatic infections of the intestinal tract. They may spread to the CNS as a rare event where they cause different neurological disease manifestations. The most typical clinical sign of TMEV infection is flaccid paralysis of hind legs. The animals appear otherwise healthy, and there is no mortality.

Experimental infection in mice provides models of poliomyelitis-like infection and virus-induced demyelinating disease including multiple sclerosis (McGavern *et al.*, 2000). After experimental infection, TMEV causes a biphasic disease in susceptible strains of mice. The acute phase is characterized by early infection of neurons in the grey matter. Encephalomyelitis may develop during this phase and may be fatal, but most animals survive and enter the second phase of the disease at 1–3 months after the acute phase. This phase

is characterized by viral persistence in the spinal cord white matter, mainly in macrophages, and leads to white matter demyelination. Persistence and demyelination occur only in genetically susceptible mouse strains while resistant strains clear the infection after early grey matter encephalomyelitis through a cytotoxic T lymphocyte response. For this reason, the nude mutation (*Foxn1*^{nu}) confers susceptibility on mice with an otherwise resistant background.

The severity and nature of disease depend on virus strain, route of inoculation, host genotype and age (Downs, 1982; Lipton and Rozhon, 1986; National Research Council, 1991; Percy and Barthold, 2001). In general, virus isolates with low virulence produce persistent CNS infection in mice whereas virulent strains are unable to cause persistent infection. Intracerebral inoculation results in the most severe infections, but the intranasal route is effective also. Experimental intracerebral infections with virulent FA and GDVII strains of TMEV are more likely to cause acute encephalomyelitis and death in weanling mice 4–5 days after inoculation ('Early Disease'). Death may be preceded by neurological manifestations of encephalitis such as hyperexcitability, convulsions, tremors, circling and rolling, and weakness. Animals may develop typical flaccid paralysis of hind limbs, and locomotion is possible only by use of the forelimbs. Interestingly, the tail is not paralysed. Experimental infections with low virulence virus strains (e.g. TO, DA, WW) are more likely to cause persistent infection with development of mild encephalomyelitis followed by a chronic demyelinating disease after a few months ('late disease'). These virus strains infect neurons in the grey matter of the brain and spinal cord during the acute phase of viral growth, followed by virus persistence in macrophages and glial cells in the spinal cord white matter. SJL, SWR, and DBA/2 strains are most susceptible to this chronic demyelinating disease. CBA and C3H/He are less susceptible strains, and strains A, C57BL/6, C57BL/10, and DBA/1 are relatively resistant (Lipton and dal Canto, 1979). Differences in humoral immune responses play a role in resistance to TMEV infection (Pena Rossi *et al.*, 1991a), but genetic factors are also important. Several genetic loci implicated in susceptibility to virus persistence, demyelination, or clinical disease have been identified, including the H-2D region of the major histocompatibility complex (Brahic and Bureau, 1998). Furthermore, the age at infection influences the severity of clinical disease. In infant mice, intracerebral infection with low virulence virus strains (e.g. TO) is often lethal. Young mice develop paralysis after an incubation period of 1–4 weeks while adult mice often show no clinical signs of infection (Downs, 1982).

The only gross lesions are secondary to the posterior paralysis and may include urine scald or dermatitis due to incontinence of urine and trauma to paralysed limbs, or wasting or atrophy of the hind limbs in long term survivors.

Theiler's murine encephalomyelitis virus infects neurons and glial cells, and histological changes in the CNS include nonsuppurative meningitis, perivasculitis, and poliomyelitis with neuronolysis, neuronophagia, and microgliosis in the brainstem and ventral horns of the spinal cord (Percy and Barthold, 2001). Demyelination in immunocompetent mice is considered to be immune-mediated. Susceptible strains develop a specific delayed-type hypersensitivity response which is the basis for inflammation and demyelination. This reaction is mediated by cytotoxic T lymphocytes (Lindsley *et al.*, 1991; Pena Rossi *et al.*, 1991b) and by the activation of cytokines as a consequence of infection of macrophages and other cells of the CNS (Rubio and Capa, 1993; Sierra and Rubio, 1993; Palma *et al.*, 2003). Protection from chronic demyelinating disease is possible by vaccination with live virus given previously by subcutaneous or intraperitoneal inoculation (Crane *et al.*, 1993; Kurtz *et al.*, 1995). Early immunosuppression at the time of infection, e.g. by treatment with cyclophosphamide or antithymocyte serum, inhibits or diminishes demyelination. Immunosuppression in mice chronically infected with TMEV leads to remyelination of oligodendrocytes (Rodriguez and Lindsley, 1992). Further details related to the pathogenesis of TMEV infections and the role of immune mechanisms have been reviewed by Yamada *et al.* (1991).

Experimental infection of *Foxn1*^{nu} mice results in acute encephalitis and demyelination. Demyelination associated with minimal inflammation and neurological signs including the typical hind limb paresis develop 2 weeks after inoculation, and most animals die within 4 weeks. In *Foxn1*^{nu} mice, demyelination is caused by a direct lytic effect of the virus on oligodendrocytes (Rosenthal *et al.*, 1986). Demyelination and lethality are reduced after administration of neutralizing antibodies (Fujinami *et al.*, 1989). Histopathological changes in *Prkdc*^{scid} mice are very similar to those in *Foxn1*^{nu} mice (Rozengurt and Sanchez, 1992).

Young mice born in infected populations usually acquire infection shortly after weaning and are almost all infected by 30 days of age. Intrauterine transmission to foetuses is possible during the early gestation period, but a placental barrier develops during gestation and

later prevents intrauterine infection (Miyamae, 1990; Abzug *et al.*, 1991).

All TMEV isolates are closely related antigenically and form a single serogroup as determined by complement fixation and HI (Lipton and Rozhon, 1986). Hemelt *et al.* (1974) demonstrated cross reactions among four strains used in experimental infections, but differences were evident in homologous and heterologous titres. The viral strain most commonly used as antigen for serological testing is GDVII. This strain agglutinates human type 0 erythrocytes at 4°C, and HI has been the standard test for routine screening of mouse populations. Meanwhile, HI has been replaced by ELISA or IFA, both of which are more sensitive and specific. Virus isolation is possible from brains or spinal cords of mice with clinical disease or from the intestinal contents of asymptomatic mice. PCR techniques also are available to test for virus-specific nucleotide sequences in biological samples (Trottier *et al.*, 2002).

Mice that have been shown to be free from TMEV by serological testing can be selected for breeding populations. If the virus is introduced into a mouse population, depopulation of infected colonies may be the most appropriate means to eliminate TMEV. Embryo transfer or caesarean derivation are the methods of choice for eliminating virus from valuable breeding populations. Foster-nursing has been reported to be effective in generating virus-free offspring (Lipman *et al.*, 1987) although transplacental transmission has been demonstrated with experimental infection early in gestation.

Lesions of demyelination in CNS of mice with clinically inapparent chronic infection may interfere with investigations that require evaluation of the CNS (Krinke and Zurbriggen, 1997). Conceivably, such lesions also could affect neuromuscular responses or coordination, and affect neurological and behavioural evaluations.

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