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Experimentally Induced Infection with Autonomous Parvoviruses, Minute Virus of Mice and H-1, in the African Multimammate Mouse (*Mastomys coucha*)

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Abstract | To determine whether the multimammate mouse (*Mastomys coucha*) could be used to evaluate rodent parvovirus-based vectors, neonates were subcutaneously inoculated with minute virus of mice (prototype strain, MVMp) or rat parvovirus H-1. The course of infection with both viruses was similar. Seroconversion occurred within two weeks after virus inoculation, as detected by use of hemagglutination-inhibition assays, and antibody titers remained high for the entire observation period of 12 months. Viral DNA and infective virions were detected in several organs of inoculated animals prior to seroconversion, as measured by use of Southern blotting and plaque assays, respectively. Infective particles subsequently became undetectable, whereas viral DNA imprints persisted in distinct organs for at least nine months. Clinical signs of parvovirus infection appeared around six weeks after virus inoculation, and consisted of hemorrhages, stunted growth, and transient hair color changes. Sudden death occurred in a significant fraction of animals infected with MVMp, but not H-1 virus, at the time of weaning. Altogether, MVMp, which is innocuous to its natural host, the mouse, and H-1 virus, which is poorly pathogenic to the rat, appear to be pathogenic for *Mastomys coucha*.

Animals from some laboratory colonies of the African multimammate mouse *Mastomys coucha* [previously assigned to the species *Mastomys natalensis* (1) and hereafter referred to as *Mastomys*] have high risk of developing benign skin tumors (2, 3). Affected colonies were found to be enzootically infected with a papillomavirus named *Mastomys natalensis* papillomavirus (MnPV), which was subsequently identified as the etiologic agent of this disease. Indeed, experimentally induced infection of virus-free animals with MnPV leads to formation of keratoacanthomas and papillomas (4–6). Thus, *Mastomys* may constitute a useful animal model of certain human epithelial neoplasms, in particular cervical and anogenital cancers that are characterized by continuous expression of distinct human papilloma virus-transforming proteins (7).

Parvoviruses are small, nuclear-replicating viruses that infect a wide variety of animal species, including humans, and typically contain a linear single-stranded DNA genome of about 5,000 nucleotides (nt) (8). Some of these viruses are of great importance as etiologic agents of animal diseases (9–15). The pathogenicity of parvoviruses can be assigned, at least in part, to their capacity for infecting and killing certain mitotically active cells. It follows that fetuses and neonates are preferential targets for parvovirus infections, although parvoviruses can occasionally cause disease in adults (9–14). Rodent parvoviruses are antigenetically related and quite similar in genome organization, size, and sequence (16–20 and references therein). Several of these parvoviruses are able to cross-infect rodents that are not their natural host (21–23). For example, Kilham rat virus (KRV) can infect *Mastomys* and induce signs and lesions that also are observed in its natural host, the rat (10, 22).

Members of the genus *Parvovirus*, hereafter referred to as parvoviruses, do not require a helper virus for their replication (8). Some of these autonomous parvoviruses, such as minute virus of mice (MVM) and H-1 virus, exert an anti-cancer surveillance in laboratory animals by inhibiting formation of spontaneous and induced (including implanted) tumors (for a review see reference 24). How parvoviruses suppress tumor formation is not fully understood at present. Because H-1 virus and MVM infect human cells, and human neoplastic cell implants in recipient mice are targets for the oncosuppressive activity of these agents (25, 26), parvoviruses deserve to be considered as potential vectors for tumor-targeted gene therapy (27–29).

Mastomys is currently used as a model for papillomavirus-induced tumorigenesis (5, 30). Parvovirus infections of rodents are often nonpathogenic (10, 15), but can complicate animal experimentation, including tumor transplantation studies (31); thus, it is of interest to characterize *Mastomys* colonies for their possible contamination with oncosuppressive parvoviruses. It is also important to determine whether *Mastomys* can be carriers of rodent parvoviruses, such as MVM or H-1 virus, to prevent these viruses from spreading to other laboratory rodents (15). Furthermore, investigation of the susceptibility of *Mastomys* to parvovirus infection is a prerequisite to use of this animal for assessment of parvovirus-based vectors. Altogether, these data prompted us to determine whether *Mastomys* can sustain infections with MVM (prototype strain, p) or H-1 viruses. Our data indicate that *Mastomys* is susceptible to infection with MVMp and H-1 viruses. The especially high sensitivity of *Mastomys* neonates to MVMp contrasts with the relative resistance of the natural host of this virus, the mouse, pointing to a preferred mode of parvovirus spreading through persistent infection devoid of overt pathogenicity.

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Materials and Methods

Animals: *Mastomys* from the breeding colony of the Deutsches Krebsforschungszentrum were kept under conventional conditions at 22°C and 55% relative humidity and 12 to 16 air changes/h. Pregnant females were transferred to isolators (Metall + Plastik, Radolfzell, Germany) and further kept with their offspring under sterile conditions at 21 to 24°C, 55% humidity, and air changes as described previously. The animals tested negative for ecto- and endoparasites, parvoviruses, and Sendai virus, but tested positive for *Pasteurella* and *Helicobacter* spp. The offspring were subcutaneously inoculated with MVMp or H-1 virus on the first day of life. Uninfected controls were kept in separate cages in the isolators. After weaning at the age of 7 weeks, the animals were kept alone or in small groups of 3 as a maximum, depending on sex, age, and size. Adult animals inoculated with high doses of virus were also housed in isolators in separate cages.

The animals were provided with a standardized mouse diet and allowed to drink water ad libitum. Infected animals were handled in a laminar flow safety cabinet (BDK Luft- und Reinraumtechnik, Sonnenbühl, Germany). All animal experiments were conducted in accordance with the institutional animal care and use committee as well with the standards set in the guidelines of the European Union.

Virus inoculation and sample collection: Virus was diluted in Dulbecco's phosphate-buffered saline without Ca^{2+} and Mg^{2+} salts and was administered subcutaneously (newborn animals) or intraperitoneally (adult animals). Neonates were inoculated with 10^3 plaque-forming units (pfu) of MVMp or 10^4 pfu of H-1 virus in a total volume of 50 µl, and 8- to 16-month-old animals were given 10^7 pfu of MVMp or 5 x 10^8 pfu of H-1 virus in a volume of 200 µl. Anesthesia of animals was induced by use of methoxyflurane (Metofane™, Janssen, Neuss, Germany), blood samples were obtained by cardiocentesis, and animals were sacrificed by cervical dislocation. Blood was frozen in liquid nitrogen for subsequent analysis of viral DNA and infective virus, and serum was prepared for serologic studies. Samples of excised organs were frozen in liquid nitrogen for detection of infective virus and viral DNA. Skin samples were taken from the area of virus inoculation.

Preparation and titration of virus: Parvovirus stocks were produced in A9 cells (32), a line of mouse fibroblasts (MVMp), or in NB cells (32), a line of SV40-transformed human kidney cells (H-1 virus), and purified on CsCl density gradients (33).

The A9 and NB cells also were used as indicator cells in plaque assays to titer MVMp and H-1 virus, respectively. Tissue culture-infective doses for 50% cell killing ($TCID_{50}$) were determined as follows. The A9 cells (MVMp) and NB cells (H-1 virus) were seeded in 96-well plates (5 x 10^2 and 10^3 cells/well, respectively), incubated overnight, and infected with serial virus dilutions. One week later, the culture medium was removed and the cells were stained with crystal violet. Titer was estimated according to the method of Reed and Muench (34); 1 $TCID_{50}$ unit corresponded to 20 pfu of H-1 virus and MVMp.

Virus recovery from tissues: Frozen tissue specimens were powdered in liquid nitrogen, suspended in 500 µl of TE buffer (50 m*M* Tris-HCl, 0. 5 m*M* EDTA, pH 8. 7) and freeze-thawed 3 times. The suspension was cleared by centrifugation at 13,000 ×*g*, and the supernatant was recovered. The pellet was re-extracted in similar manner, and the supernatants were combined and titered.

Hemagglutination-inhibition assay: Virus hemagglutination-inhibition (HAI) titer of serum obtained from animals was determined at 4°C in microtitration plates, using 0. 33% guinea pig erythrocytes and 8 hemagglutinating units of H-1 virus and MVMp. Samples were diluted in Dulbecco's phosphate-buffered saline. Serum from MVMp-infected *Mastomys* was treated with 100 U of receptor-destroying enzyme/ml (BioWhittaker, Walkersville, MD) prior to use. Serum was heat inactivated for 30 minutes at 56°C and subsequently assayed for HAI activity.

Extraction of viral DNA and Southern blot analysis: Viral DNA was extracted by use of a modification of the procedure described by McMaster et al. (35). In short, the homogenized tissue was incubated overnight at 50°C in digestion buffer (100 m*M* NaCl, 10 m*M* Tris-HCl, 25 m*M* EDTA, 500 mg of proteinase K/ml, and 1% (wt/vol) sodium dodecyl sulfate, pH 8. 0). High-molecular weight DNA was precipitated with NaCl (final concentration, 1*M*), centrifuged (13,000 ×*g* at 4°C), and the viral DNA in the supernatant was ethanol-precipitated at -70°C. The precipitate was taken up in 10 m*M* Tris-HCl (pH 8. 0) and 5 m*M* EDTA, treated with RNase A (0.2 mg/ml, 37°C, 30 minutes), and proteins were removed by extraction with phenol/chloroform/isoamyl/alcohol (25:24:1). Viral DNA was recovered by ethanol precipi tation, dissolved in 10 m*M* Tris-HCl (pH 8. 0) and 5 m*M* EDTA, fractionated by 1% agarose gel electrophoresis, and blotted onto nylon membranes (Hybond N+, Amersham Int., Buckinghamshire, UK). Viral DNA was visualized by hybridization with MVMp or H-1 virus DNA probes that were ^{32}P-labeled, using the Megaprime™ DNA labeling kit (Amersham Pharmacia Biotech, Freiburg, Germany) according to the manufacturer's recommendations, and the filters were exposured to x-ray film. The DNA samples for polymerase chain reaction (PCR) analysis were prepared, using the QiaAmp™ Tissue Kit (Qiagen, Hilden, Germany) according to the recommendations of the manufacturer.

Polymerase chain reaction analysis: The primer sets TCA ATG CGC TCA CCA TCT CTG and TCG TAG GCT TCG TCG TGT TCT (H-1 virus), or ACG CTC ACC ATT CAC GAC ACC GAA A and ATC ATA GGC CTC GTC GTG CTC TTT G (MVMp) flank a 515- or 511-nt sequence from the 5' end of the VP region of H-1 virus and MVMp DNA, respectively. The PCR analysis was performed using the GeneAmp™ PCR reagent kit with AmpliTaq™ DNA polymerase (Perkin Elmer, Weiterstadt, Germany) according to purchaser's recommendations. After an initial denaturation step (4 minutes at 94°C), 35 cycles consisting of 1.5 minutes at 94°C, 1.5 minutes at 64°C (MVMp) or 60°C (H-1 virus), and 2 minutes at 72°C were performed, followed by a final elongation step at 72°C for 7 minutes. The reaction products were electrophoresed at 8 V/cm on 1% agarose gels containing ethidium bromide and were examined under UV-light for the amplified sequence. The gels were capillary blotted to a positively charged nylon membrane (Hybond N+, Amersham International, Buckinghamshire, UK), hybridized with specific probes, and autoradiographed.

Histologic examination: For histologic examination, animals were sacrificed as described previously and excised organs (liver, lung, heart, spleen, kidney, and skin) were fixed in 4% formaldehyde. Fixed organ specimens were embedded in paraffin, sectioned at 5-µm thickness, and stained with hematoxylin and eosin.

Results

Recovery of infective virus: Newborn *Mastomys* were infected with MVMp or H-1 virus, and production of infective vi-

rus was determined by use of $TCID_{50}$ assays (Figure 1). Substantial amounts of virus could be recovered from infants between Postinfection days (PID) 2 and 20. The MVMp (panel A) could first be detected in the skin at PID 4. The highest amounts of MVMp virus were recovered on PID 8, ranging from 7.5×10^4 $TCID_{50}$/ml blood to 3.3×10^9 $TCID_{50}$/g of skin. Infective H-1 virus (panel B) could be detected as early as PID 2 in the skin, lungs, and heart. The H-1 virus amounts were highest in the kidney at PID 14 ($6.3 \times 10^6 TCID_{50}$/g) and in the heart and skin at PID 8 (2.4×10^6 and 1.7×10^5 $TCID_{50}$/g, respectively). Virus titer dropped at later times (panel A, PID 20) and became extremely low or undetectable by one month after infection and later. For instance, at 4 weeks after infection, infective H-1 virus could not be recovered from heart and lungs and only low amounts of virus (expressed as pfu/10 mg of tissue) could be detected in liver (25), kidneys (2.5), spleen (62), and skin (45) (and data not shown). Comparable low doses of H-1 virus were recovered from a two-month-old animal that was found dead with 17 (lung), 37 (liver), 112 (spleen) and 15 (skin and kidney) pfu/10 mg of tissue. The time-dependent increase in the amounts of recovered virus indicated that virus production took place in infected animals. Yet, virus could only be detected during a short period, suggesting that antiviral responses may develop. In contrast with those from infected animals, extracts from untreated controls did not cause killing of NB indicator cells (data not shown). Therefore, the *Mastomys* colony did not appear to undergo a measurable persistent infection with parvovirus H-1 and MVM.

Viral DNA replication: To ascertain that infective virus that could be recovered from various organs was locally produced and did not originate from other tissues, Southern blotting was performed to identify viral DNA replicative forms (RF). The MVMp monomer (mRF), and to a lower extent dimer RF (dRF) DNA species were detected in various organs at several time points (Figure 2 and data not shown). In the skin area comprising the virus inoculation site, mRF could be detected at PID 2 (Figure 2A, lane 7) whereas this intermediate appeared later at the distant sites (Figure 2B and 2C). Replication of MVMp DNA peaked between PID 8 and 15. At PID 8, viral DNA mostly consisted of the mRF species (Figure 2B), whereas at PID 15, many organs contained mostly single-stranded (ss) progeny DNA (Figure 2C). The amounts of viral ss and RF DNA isolated from the various organs were in rough proportion to the respective yields of virus recovered from these organs. Yet, viral DNA persisted longer than viral particles, since at later times when no infective virus could be detected anymore in the skin (PID 35), viral DNA was still visible in Southern blots (data not shown). Substantial but lower amounts of mRF, compared with those in MVMp-infected animals, could be detected PID 2 in the skin and PID 8 in the heart of H-1 virus-infected *Mastomys* (data not shown). No DNA species were detected in control animals that would be indica-

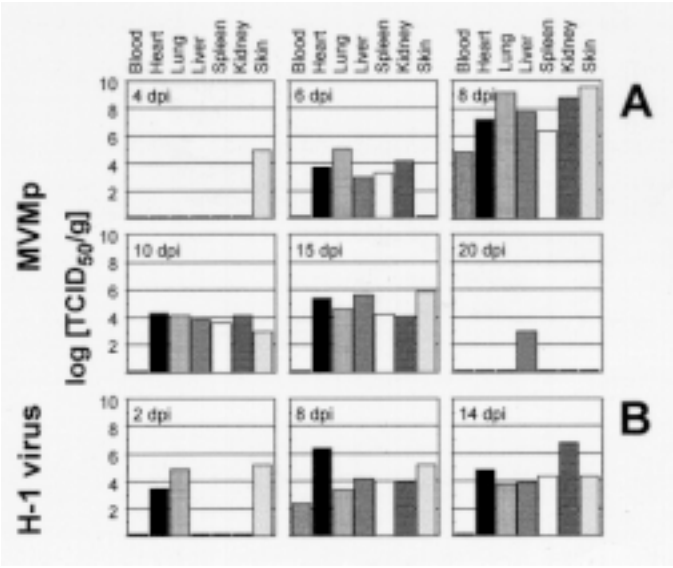

Figure 1. Recovery of infective minute virus of mice strain p (MVMp) and H-1 virus from *Mastomys coucha* (*Mastomys*) after neonatal infection with 10^3 plaque-forming units (pfu) of MVMp **(A)** or 10^4 pfu of H-1 virus **(B)**. Animals were killed at various days after infection, and indicated organs were processed for virus titration by 50% tissue culture-infective dose ($TCID_{50}$) assays. Virus titer is given as logarithm of the $TCID_{50}$ titer per gram of tissue.

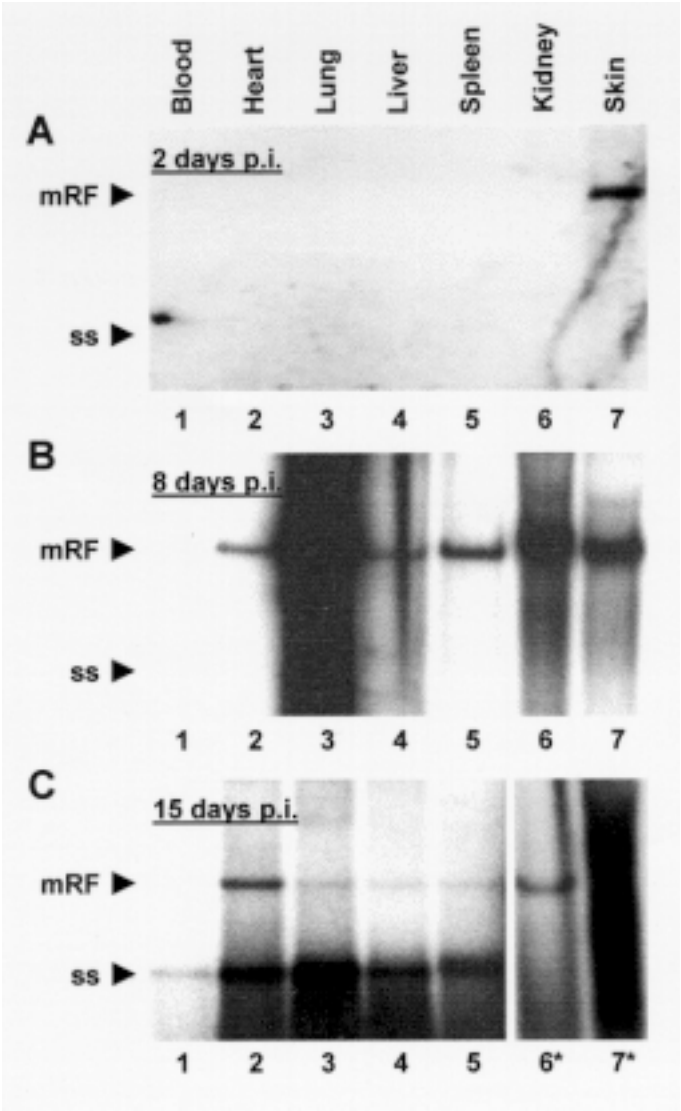

Figure 2. Replication of MVMp DNA in various *Mastomys* organs 2 **(A)**, 8 **(B)**, and 15 **(C)** days after neonatal infection. Positions of single-stranded genomic DNA (ss) and monomeric replicative forms (mRF) are indicated.

tive of a spontaneous or persistent infection of Mastomys with H-1 virus, MVM, or related parvovirus.

Antiviral humoral immune response: Serum was obtained at intervals from *Mastomys* infected as newborns and was analyzed for anti-viral antibodies by use of HAI assays. As indicated (Figure 3), infected animals had a biphasic antibody response against MVMp and H-1 virus. A primary clear-cut (MVMp) or faint (H-1 virus) antibody response took place within the first week after infection. After a decrease in antibody titer around PID 10, HAI values increased again and reached plateaus in a few days with titer > 1:1,280 and 1:640 for MVMp and H-1 virus, respectively. For at least one year after neonatal inoculation of virus, these titers persisted at an unchanged high value in all MVMp-infected animals tested (n = 10) and at various, often decreased values in H-1 virus-infected animals (n = 12) (Figure 3, right side).

Virus persistence: Tissue extracts from clinically healthy 9- to 12-month-old *Mastomys* that had been mock-treated or infected with MVMp or H-1 virus, were analyzed for viral DNA and infective virus by use of PCR analysis and plaque assay, respectively. Several organs of MVMp-infected animals, in particular the heart, lungs, kidneys, and skin, gave signals corresponding to approximately 1 pg of co-processed MVMp-DNA (Figure 4A). The liver gave a much weaker signal, and viral DNA could not be detected in the spleen (Figure 4, lanes 3 and 4). The organ distribution of viral DNA in H-1 virus-infected *Mastomys* was slightly different from the pattern observed in MVMp-infected animals. Substantial amounts of H-1 viral DNA could only be detected in the heart and lungs (Figure 4B); the liver and skin gave faint signals, whereas the spleen and kidneys tested negative. The H-1 virus-generated signals were in general weaker than those of MVMp, indicating that H-1 DNA persisted at a lower amount than that of MVMp DNA in the target organs. No H-1 virus- nor MVMp-specific DNA could be found in the organs of uninfected animals (Figures 4A and 4B). Although parvoviral DNA persisted in several organs of infected animals for more than nine months, we were not able to detect infective viral particles by use of plaque assays at these later times (data not shown).

Clinical observations: Neonatal MVMp- and H-1 virus infections were associated with the appearance of morphologic aberrations. Hair color changes and growth retardation were the most frequent alterations that were observed in treated animals, whereas none of the non-infected animals developed these lesions (Table 1 and Figure 5). Among littermates, animals with white and gray fur were seen in addition to those with normal-colored fur. Moreover, areas with reduced hair density and even baldness were occasionally observed in some infected juveniles (Figure 5, panel A, animal 2, and panel B). These alterations of the fur disappeared with time.

Growth disturbances were frequent among H-1 virus- and MVMp-infected *Mastomys* (panel A, animal 2; panel B, animal 3). To quantify this effect, males and females were separately evaluated at the age of 9 weeks (Figure 6). Due to virus-induced mortality, animals from the MVMp-infected group were strongly reduced in number. Despite this shortcoming, mean body weight of MVMp-infected males was significantly lower than that of uninfected males (Welch test: $\alpha 2 < 0.0005$). Compared with that of control males, body weight of H-1 virus-infected males also was reduced but to a lesser extent than that for MVMp $\alpha/2 < 0$.

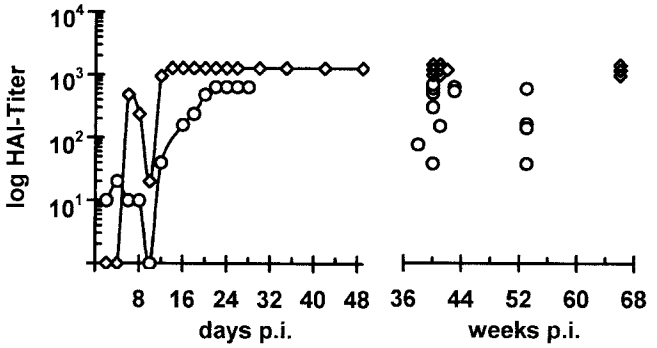

Figure 3. Humoral immune responses of *Mastomys* to neonatal infection with MVMp (rhombi) or H-1 virus (circles). Antibody titer was determined at early (left) and late (right) times after infection by use of the hemagglutination inhibition (HAI) test, using serial dilutions of *Mastomys* serum. Data are average values from 2 to 3 animals (left panel) or results from single animals (right panel).

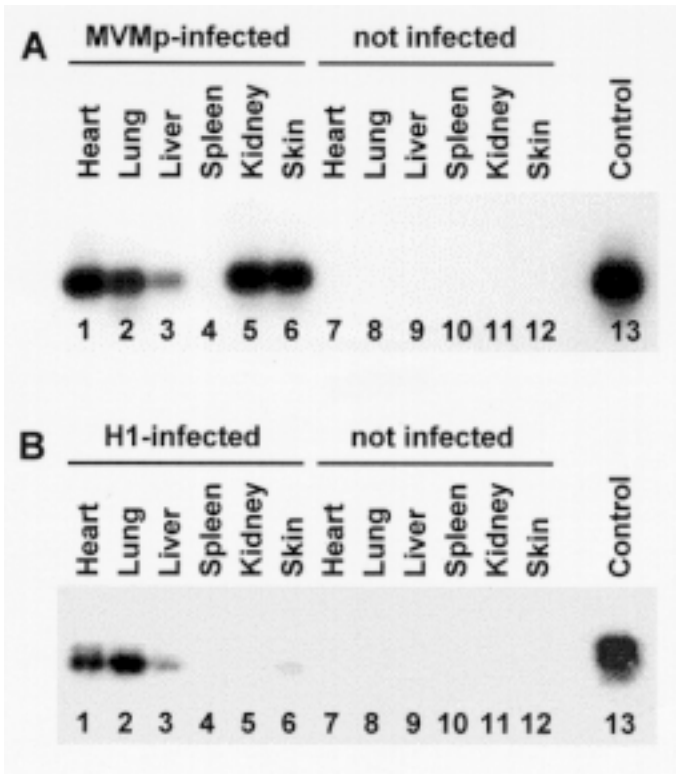

Figure 4. Persistence of MVMp and H-1 virus DNA in organs of neonatally and mock-infected *Mastomys*. The DNA was isolated from indicated organs of MVMp- **(A)**, H-1 virus- **(B)**, or mock-infected animals at 12, 10, and 9 months after infection, respectively. One picogram of co-processed cloned MVMp DNA (A) or H-1 virus DNA (B) was used as standard (lanes 13).

Table 1. Changes in the appearance and behavior of *Mastomys coucha* (*Mastomys*) at six weeks after being neonatally infected with minute virus of mice (MVMp) or H-1 virus

	MVMp (n = 47)	H-1 (n = 49)	Control (n = 50)
Growth retardation	11	8	0
Altered hair color	5	17	0
Reduced hair density	1	3	0
Hunched appearance	3	0	0

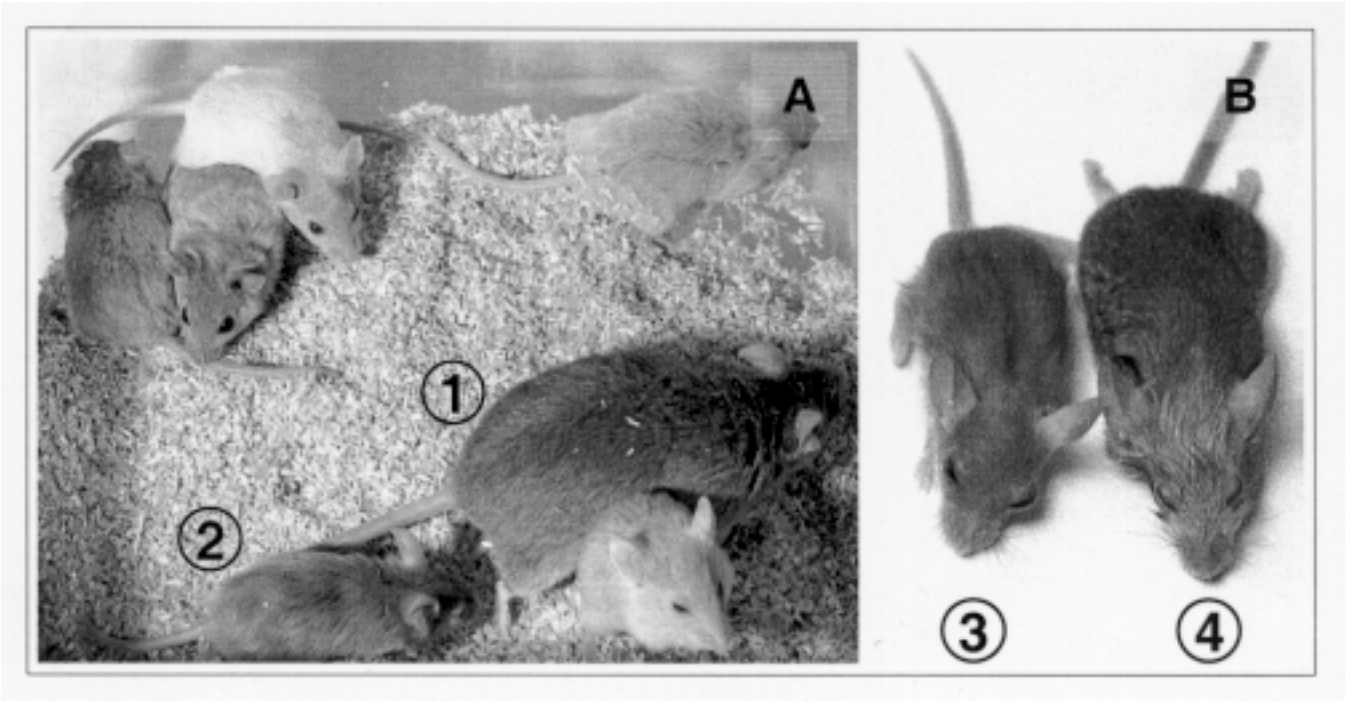

Figure 5. Virus-induced morphopathologic changes in neonatally MVMp- and H-1 virus- infected *Mastomys*. **(A)** The MVMp-induced hair color changes in a 7-week-old litter of *Mastomys* in comparison with the hair color of the untreated mother (animal No. 1). Animal 2 has reduced hair density on the back. **(B)** Reduced growth (animal 3) and hair density (animal 4) in 7-week-old H-1 virus-infected *Mastomys* siblings.

05). Significant mean body weight reduction was not observed in the groups of MVMp- or H-1 virus-inoculated females, although some infected individuals were clearly growth retarded, compared with controls. Besides the aforementioned aberrations, a few MVMp-infected animals, but not neonatally H-1 virus-inoculated animals, became severely ill, manifesting lethargy and hunched appearance (Table 1).

Deaths were recorded over a period of more than one year (Figure 7). A few infected animals were found dead or cannibalized within the first two weeks after virus infection. This was in all likelihood unrelated to infection since neonatal death and cannibalism also happened occasionally in untreated *Mastomys* (unpublished observations). As indicated (Figure 7), > 60% of MVMp-infected *Mastomys* died between the sixth and tenth week after infection, coinciding with the period in which the juveniles were weaned. Mortality was less pronounced in H-1 virus-infected animals, among which only 8% died between the fourth and fourteenth week after infection. These delayed deaths were likely due to virus infection, as none of the untreated animals died during that period.

It also was determined whether adult animals were susceptible to parvovirus H-1 and MVMp infections. The adult *Mastomys* (n = 8) that were subcutaneously inoculated with a high-dose of H-1 virus (5×10^8 pfu) at the age of 8 to 13 months, were killed at 2 weeks after infection. They all developed slight to mid-grade erythropenia and the leukocyte count reflected anything from leukopenia to leukocytosis. Only one of these animals developed clinical disease characterized by lethargy, weight loss and hypothermia. The infection of 6 adult *Mastomys* with 10^7 pfu of MVMp induced effects similar to those of H-1 virus (erythropenia, leukopenia, and leukocytosis) and one animal died at PID 14.

Morphopathologic changes: Postmortem examination of *Mastomys* that died as a consequence of MVMp or H-1 virus infection at birth often revealed similar lesions. From nine MVMp-infected animals that succumbed, all suffered from hemorrhages in the intestine (8) and/or lungs (4) and five had clearly smaller spleen than that of two-week-old control animals (data not shown). A few animals that died more than one year after infection also had intestinal hemorrhages and occasionally hepatopathy. Similarly, among neonatally H-1 virus-infected *Mastomys* (n = 12), a substantial number had intestinal hemorrhages (4), pulmonary, pleural and peritoneal hemorrhages (1), hepatopathy (2), and pneumonia (8) when necropsied 14 to 20 months after infection (data not shown).

As stated previously, of eight adult *Mastomys* that were injected with high amounts of H-1 virus, only one developed clinical disease. Postmortem examination of this animal also revealed intestinal hemorrhages and hepatopathy, whereas the other injected individuals had no gross lesions.

Histopathologic changes: In contrast to the frequent morphopathologic alterations observed in neonatally MVMp-infected *Mastomys* that died between 6 and 10 weeks after infection, only a few histopathologic aberrations could be detected in these animals. Histologic alterations were observed in animals that were sacrificed several months to one year after MVMp or H-1 virus infection and in adult animals shortly after inoculation of a high dose (5×10^8 pfu) of H-1 virus (Table 2). These alterations were predominantly in the liver and lungs. Fatty metamorphosis had developed in the liver of several neonatally MVMp-infected animals (Table 2 and Figure 8, panel A), and the liver of one adult H-1 virus-infected animal was infiltrated with lymphocytes. In several animals, we observed various degrees of necrosis of single hepatocytes, irrespective of whether they were infected with MVMp or H-1 virus as newborns or as

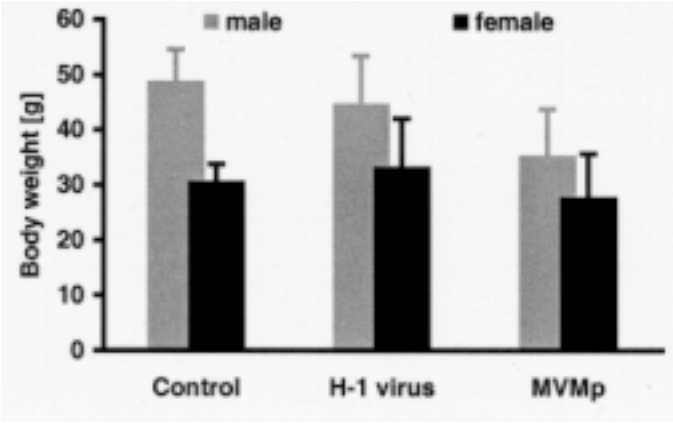

Figure 6. Body weight of 9-week-old, sex-matched *Mastomys* that were neonatally mock infected (n_{male} = 23; n_{female} = 27) (control) or infected with MVMp (n_{male} = 14; n_{female} = 11) or H-1 virus (n_{male} = 26; n_{female} = 22). Standard deviations are indicated by vertical bars.

adults (Table 2, Figure 8, panel A). In a few instances, single hepatocyte necrosis had developed to focal liver necrosis (Table 2 and Figure 8, panel B). Moreover, the lungs of H-1 virus-infected animals were more often affected by bronchopneumonic infiltrations than were those of controls, and MVMp and H-1 virus infection caused pulmonary lymphocytic infiltrations (Table 2).

Discussion

On subcutaneous inoculation into newborn *Mastomys coucha*, MVMp and H-1 virus replicate and cause clinical effects. Our data unequivocally indicate that *Mastomys* is susceptible to the mouse (MVMp) and the rat (H-1) parvovirus. It is known that KRV, another rat parvovirus, also can infect *Mastomys* (22). The fact that *Mastomys* can be a host for all three parvoviruses may reflect the close taxonomic relationship between *Mastomys*, mice, and rats. The DNA replicative intermediates and infective particles could be detected first (i. e., within PID 2 to 4) in the skin area in which the input virus was inoculated, before they were found in various other tissues. This is indicative of a biphasic infection that involves initial virus replication at the port of entry (skin) prior to spread and further multiplication of progeny particles at secondary sites. Occasional detection of infective H-1 virus in the heart and lungs concomitantly with its appearance in the skin can probably be assigned to accidental injection of virus into a blood vessel. Under natural conditions, most parvoviruses are thought to enter the host through the oronasal route (10). Indeed, after intranasal infection of BALB/c mice with MVMi (the immunosuppressive strain of MVM), infective virus and replicating viral DNA were detected within two days after infection, not only in the lungs, but also in several organs of the hematopoetic system (36). In the present experimental model, H-1 virus and MVMp initially replicated to similar extents in the inoculated skin area. Yet, after subsequent dissemination to other organs, MVMp multiplied more efficiently than did H-1 virus, resulting in a 10^2- to 10^3- fold higher overall production of the former virus. The production of MVMp was 10^2 to 10^4 times higher in *Mastomys* than in intranasally infected C3H mice, in which virus titer reached only 10^3 to 10^4 pfu/g of tissue (37). Therefore, *Mastomys* can be considered a highly susceptible host to MVMp, but only moderately susceptible to H-1 virus.

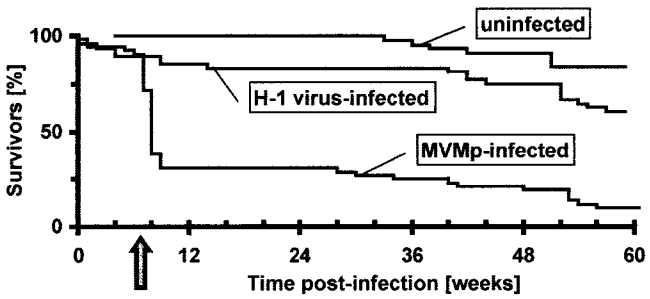

Figure 7. Survival of neonatally-infected *Mastomys* with time, expressed as percentage of the initial number of animals. Deaths include animals that died spontaneously or diseased animals that had to be euthanized for ethical reasons. The arrow indicates the time at which the animals were weaned. Data from 52 (MVMp), 48 (H-1 virus), and 44 (mock) infected animals.

Table 2. Histopathologic alterations in MVMp- and H-1 virus-infected *Mastomys*

Virus	MVMp		H-1		Control
Infection mode	nb	ad	nb	ad	-
n	11	6	13	8	9
Lung					
Lymphocytic infiltration	4	2	4	3	0
Bronchopneumonic infiltration	0	0	4	3	1
Liver					
Fatty metamorphosis	2	3	0	0	0
Lymphocytic infiltration	0	0	0	1	0
Single hepatocyte necrosis	2	4	3	3	0
Focal necrosis	1	0	0	1	0
Extramedullary hematopoiesis	0	4	7	2	0

nb = newborn; ad = adult; n = number of animals.

The immune response to neonatal MVMp or H-1 virus infection is typically biphasic. Although the HAI assay used cannot distinguish between IgM and IgG, we assume that the first HAI peak results from production of IgM, whereas the second increase in HAI activity and the following plateau phase are attributable to IgG. However, parvoviruses are known for their ability to resist full eradication from infected organisms by the immune system, and to persist in a form allowing their reactivation under specific physiologic conditions (38). This is the case in newborn or juvenile rat infection with KRV, which invariably results in virus persistence in the presence of host immunity (38, 39). The long persistence of some autonomous parvoviruses in host tissues could also account for the finding that neonatal infection with H-1 virus reduced the incidence of spontaneous and induced tumors in adult hamsters (21). The state in which parvoviruses persist is still elusive, yet maintenance of high antibody titer indicates that viral gene expression, or even virion production, probably takes place to some extent, either continuously or recurrently.

Coexistence of immunity and persisting viruses is, therefore, a characteristic feature of parvovirus infections. This is further exemplified by results of the study reported here indicating that replicating viral DNA was still present in various organs several months after neonatal infection of *Mastomys*. Although infective virus could not be detected anymore at those late stages, the viral life cycle can be assumed to proceed at least in part in certain cells for a long time after animal infection, since *Mastomys* inoculated at birth had lifelong maintenance of high titer of antiviral antibodies and virus-induced clinical signs of infection. It remains to be determined whether tissues serving as parvovirus

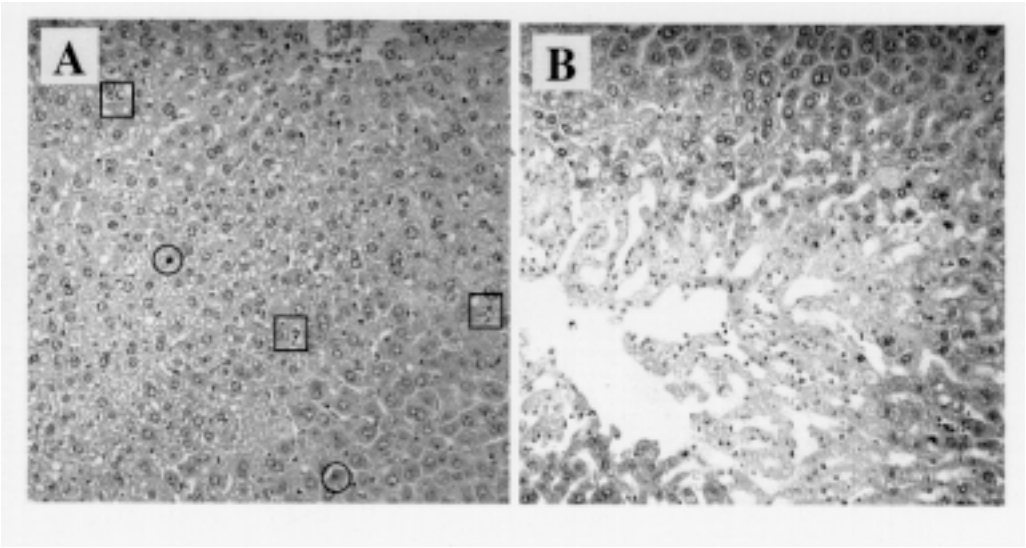

Figure 8. Histopathologic features in the liver of MVMp- and H1 virus-infected *Mastomys*. H&E stain; magnification = 140x. **(A)** Liver of clinically inconspicous female sacrificed 12 months after neonatal MVMp infection showing single hepatocytes with condensed nuclei undergoining necrosis (circles) and hematopoetic foci (squares). **(B)** Focal necrosis showing various stages of hepatocyte degeneration from a diseased 9-month-old male euthanized 3 weeks after high-dose H-1 virus injection.

reservoirs harbor isolated viral nucleic acid and protein constituents or occasionally sustain full particle production and possibly also direct cell-to-cell transmission sheltered from the immune system. Such a low level or intermittent production of virus may have escaped detection in our assays.

Although H-1 virus and MVMp differed in the severity of the disease they induced in *Mastomys*, both viruses had qualitatively similar pathological features. Some of the disturbances caused by H-1 virus and MVMp in *Mastomys*, in particular growth retardation and hepatitis, are reminiscent of the previously described effects of KRV in this animal (22). It is worth noting that siblings among a treated litter had a varying sensitivity to MVMp and H-1 virus infection, which may be due, at least in part, to differences in their respective capacities for mounting an antiviral immune response.

Growth retardation is prevalent in animals that are infected with autonomous parvoviruses in utero or as neonates (10). For example, injection of newborn hamsters with parvovirus H-1 induces the so-called osteoblastic syndrome, which is characterized by mongoloid dwarfism (40–42). Neonatal infection of *Mastomys* with MVMp and, to a lesser extent, H-1 virus also resulted in a appreciable retardation of the growth of treated animals. Another distinct feature of neonatal infection of *Mastomys* with MVMp and H-1 virus consisted in the fur variegation and reduced hair density observed among siblings, pointing to a cytopathic effect of the parvoviruses on cells of hair follicles. This alteration was transient, and animals surviving the infection recovered normal fur at later stages. The developing immune response in inoculated animals may restrict virus dissemination within the skin allowing uninfected cells to sustain normal hair growth. To the authors' knowledge, the parvovirus-induced fur alterations revealed by results of this study had not been documented previously in other rodent species, although parvovirus infection of hair follicles has been reported in newborn ferrets (43), and are reminiscent of changes induced by avian parvoviruses in the plumage of geese and chicken (12).

The MVMp infection caused the death of a substantial proportion of infected animals between 6 and 10 weeks after infection. This was somewhat unexpected since the acute phase of infection had already been overcome by the immune response at that time, and infective virus could not be recovered from dying animals. The reason for the time lag between virus infection and acute disease outbreak is presently unclear. It was noticed that mortality of neonatally MVMp-infected *Mastomys* appeared at the time of weaning. This coincidence raises the possibility that this may be a period of physiologic perturbation (e. g., stress-induced immune suppression) enhancing the animal's susceptibility to parvovirus replication and/or cytopathic effect. Moreover, the loss of maternal antibody may have contributed to the death of these animals.

Irrespective of the mode of action of MVMp and H-1 virus in *Mastomys*, the study reported here provides information on target organs. Prime candidates consist of the intestine and, to a lower extent, the lungs, since most *Mastomys* animals succumbing to MVMp infection had hemorrhages in these organs. Intestinal hemorrhages constitute a hallmark of parvovirus-induced disease in rodents (10, 15). There is evidence to suggest that the higher pathogenicity of MVMi, versus MVMp in C3H mice depends on tropism of the former virus for mouse endothelial cells (44). It is, therefore, conceivable that MVMp is endowed with endotheliotropic properties in *Mastomys*, although other (including indirect) viral effects leading to hemorrhages also should be considered.

The liver constitutes another target for MVMp and H-1 virus infection in *Mastomys*, as it figured among the organs with the highest virus DNA and particle contents in dying animals. Furthermore, the liver of diseased *Mastomys* had distinct pathologic alterations, including focal necrosis and fatty degeneration. Similarly, the liver was reported to be a target for rat, hamster, and goslings infection with KRV, H-1 virus, and goose parvovirus, respectively (10, 12, 41, 45–47). Besides focal necrotic areas that are likely to play a role in morbidity, the liver of diseased MVMp or H-1 virus-infected *Mastomys* contained isolated necrotic hepatocytes, which may contribute to virus persistence. It remains to be determined, however, whether the death of single hepatocytes is a direct result of parvovirus replication.

The hematopoetic system also appeared to be perturbed in several respects in consequence of MVMp or H-1 virus infection of *Mastomys*. Red and white blood cell counts were affected to various degrees, suggesting that hematopoetic progenitor cells may be targets for parvovirus infection in *Mastomys*. This contention remains to be proven, but is in keeping with the myeloid depression observed after newborn mice infection with the

lymphotropic virus strain MVMi (36). In general, blood cell count anomalies constitute a typical feature of parvovirus infections in several animal hosts (10), and are considered a prognostic marker of infection of cats with autonomous parvoviruses (13). In this study, extramyeloid hematopoetic foci were detected in the liver of several infected *Mastomys*, and may represent attempts of compensating from impaired bone marrow function. This stresses the importance of testing rodent colonies for contamination with parvoviruses, which may be not clinically apparent but still interfere with experimentation, in particular due to changes in the hematopoetic and immune system. Furthermore, infiltration of liver and lungs with inflammatory cells took place in MVMp- and H-1 virus-infected *Mastomys*.

The H-1 virus is well known as a pathogenic agent in rats (10), whereas MVMp is considered nonpathogenic in mice (37, 48). Our data indicate that MVMp is more pathogenic in *Mastomys* than is H-1 virus after subcutaneous inoculations. Neonatal MVMp and H-1 virus infections are hardly a problem in *Mastomys*-keeping animal facilities, because the viruses cause clear morphologic alterations and lethal disease, making the infection apparent. A possible problem may lie in latent infection of adult animals, as it may affect the hematopoetic and immune system and, thus, interfere with animal experiments. The high pathogenicity of MVMp and H-1 virus for *Mastomys* render this animal less appropriate as a model to evaluate parvovirus-mediated anti-tumor therapies, in particular as we cannot actually guarantee that the recombinant parvovirus stocks are free of replication-proficient virus (28, 29). As *Mastomys* animals are susceptible to the widespread rodent parvoviruses H-1 virus and MVM, it should be ascertained that animals used for research purposes are free from these or related viruses.

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