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The Pathogenesis of Infection with Minute Virus of Mice Depends on Expression of the Small Nonstructural Protein NS2 and on the Genotype of the Allotropic Determinants VP1 and VP2

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Neonatal C3H/He mice were oronasally inoculated with similar doses of four genotypes of minute virus of mice (MVM). MVMp, a fibroblast-specific variant, caused an asymptomatic infection. MVM(1035), a chimera which had the allotropic determinant of virulent MVMi inserted onto an MVMp background, caused a lethal infection and renal papillary infarcts, the hallmark of MVMi infection. MVMi(NS2-1990), the virulent lymphocyte-specific variant mutated to eliminate NS2 synthesis, was infectious but caused an asymptomatic infection. Sequential virus titration, histology, in situ hybridization with a full-length MVMi genomic probe, and immunohistochemistry for viral capsid antigen were used to compare the pathogenesis of infection with the four MVM genotypes. Infectious virus was recovered from multiple organs of mice infected with MVMi, MVMp, and MVM(1035) but not from mice infected with MVMi(NS2-1990). MVMp titers were lower than MVMi titers in all organs except the intestine. MVM(1035) titers were higher than MVMi titers in all organs except the blood. MVMp was localized to connective tissue elements of the intestine, to cells in mesenteric lymph nodes, and rarely to cells in other organs. MVM(1035) was localized to multiple organs and shared the same target cells, endothelium, lymphoid cells, and hematopoietic cells, as MVMi. MVM(1035) also replicated in external germinal cells of the cerebellum and smooth muscle cells of the stomach and colon, which were not targets of MVMi or MVMp infection. MVMi(NS2-1990) replicated to a limited degree in some MVMi target organs.

The autonomous murine parvovirus minute virus of mice (MVM) contains a 5.1-kb, linear, single-stranded DNA genome that encodes only four translation products: two nonstructural proteins, NS1 and NS2, and two capsid proteins, VP1 and VP2 (2, 7–9). To replicate, MVM requires cellular factors that are transiently expressed during the S phase of the cell cycle (3, 25, 27) and other cellular factors that are expressed during differentiation (3, 19, 24). Two serologically indistinguishable strains of MVM, termed allotropic variants, require different differentiation-dependent factors for their replication in vitro. MVMp, the prototype strain, productively infects cells of fibroblast origin (24, 26). MVMi, a variant strain, productively infects T cells (4, 17). MVMp and MVMi are 97% homologous at the nucleotide level (2). The fibrotropic determinant maps to a 236-nucleotide fragment of the genome of MVMp in the region of the capsid gene common to both VP1 and VP2 (1, 12). The lymphotropic determinant maps to an 820-bp region of the overlapping capsid gene of MVMi that encompasses the fibrotropic determinant (12). The 83-kDa NS1 is a stable nuclear phosphoprotein that is required for viral DNA replication as well as for *trans* activation of the P38 promoter (7, 9–11, 21). The 25-kDa NS2 is a labile phosphoprotein that shares its first 85 amino acids with NS1. Little is known about the function of NS2. It is required for efficient virus production and viral DNA replication in cells from the natural host species but not from heterologous species (16, 20). The natural host requirement for NS2 has also been reported in vivo (16).

MVMi causes a lethal infection in perinatal mice of susceptible strains (5, 13) whereas MVMp infection is inapparent (13). After oronasal inoculation, MVMi causes a generalized infection primarily of endothelium, lymphocytes, and hematopoietic cells and produces bilateral infarcts of the renal papilli (5). The course of avirulent MVMp infection in neonatal mice has not been characterized in detail. MVMp has been reported to remain confined to the oropharynx of neonatal mice after oronasal inoculation (13). The genetic determinants of MVM pathogenicity have not been investigated.

The purposes of this study were to (i) characterize apathogenic MVMp infection in perinatal mice and compare it with pathogenic MVMi infection, (ii) determine the effect on perinatal infection of replacing the allotropic determinant of MVMp with that of MVMi using an infectious intertypic recombinant, and (iii) determine whether failure of MVMi to express NS2 alters its pathogenesis in neonatal mice, using an NS2 mutant.

MATERIALS AND METHODS

Mice. Adult C3H/HeSnJ mice were obtained from Jackson Laboratory, Bar Harbor, Maine. Male and female mice were pair housed in Micro-isolator cages (Lab Products, Maywood, N.J.), using husbandry practices that guarded against infection with common murine infections including MVM and that have been described previously (5). Mice were free of infection with MVM at the time of inoculation as deter-

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mined by routine serological monitoring of the dams. Because of the strong influence of host age on MVM infection, dams were observed twice daily for parturition and care was taken to ensure that mice were infected within 24 h of birth.

Virus strains and infectivity titrations. A clonal isolate of MVMi (26), originally recovered as a contaminant of EL4 lymphoma cells (4), was passaged twice in S49 ITB2 cells (BALB/c lymphoma cells). A cleared lysate was used as the virus stock.

The cloning of MVMp by terminal dilution in A9 ouab^{r}1 1 cells has been described previously (25). A cleared lysate was used as virus stock.

MVM(1035) was produced from a chimeric MVM plasmid, pPI1035, the construction of which has been described elsewhere (12). Briefly, the fragment of MVMi between the *Eco*RI and *Xba*I restriction sites (nucleotides 3522 to 4342) was cloned into the parent plasmid, pMM984, which contained an infectious MVMp genome, by way of several plasmid intermediates. Infectious virus was produced by calcium phosphate transfection of Hyb 1/11 cells (18). The identity of the clone was confirmed by restriction endonuclease and fragment-length analysis. Virus was not passaged prior to use.

Construction of MVMi(NS2-1990) was essentially as described previously for the analogous mutant of MVMp (20). The MVMi *Hae*III fragment (nucleotides 1854 to 2378), cloned into M13mp19, was used as mutation template together with a mutagenic oligonucleotide of MVMi sequence for site-directed mutagenesis (15). The oligonucleotide introduced an A-C transversion at nucleotide 1990, which disabled the MVM R2 large splice acceptor site, thus preventing NS2 production, while leaving NS1 unaltered. After mutagenesis, the mutant MVMi *Bst*EII-*Xho*I fragment (nucleotides 1886 to 2072) was rebuilt back into the infectious clone of MVMi, and the inserted fragment was completely sequenced to ensure the absence of additional mutations. The mutant clone was transfected onto NB324 K cells, and infectious virus was released by standard freeze-thawing. Virus was not passaged prior to use.

BHK-21 cells were used for primary isolation and titration of MVMi, MVMp, MVM(1035), and MVMi(NS2-1990). Tissue homogenates (1 to 10%, wt/vol) were screened for infectious virus by fluorescent focus assay (23). An enzyme immunoassay with sensitivity identical to the fluorescent focus assay was used to quantify virus in tissue homogenates (23). Titers were expressed as fluorescent focus-forming units or median infectious doses (ID_{50}) per gram of tissue. The limit of detection was approximately 100 focus-forming units per g. BHK cells were equally permissive for MVMi, MVMp, and NS2 mutants of MVMp as described elsewhere (20, 23). Titrations of virus stocks indicated that this applied to MVM(1035) and the NS2 mutant of MVMi as well.

Mouse inoculations and necropsies. Mice less than 24 h old were inoculated oronasally with approximately 10^3 focus-forming units of each virus in 5 μl. This dose was selected because it was the highest common dose that could be attained for the four MVM genotypes. This dose was infectious for the four genotypes as judged by the appearance of convalescent serum antibodies in mice infected with avirulent strains and by mortality with typical histopathological changes in mice infected with virulent viruses. The necropsy methods and tissue fixation have been described previously (5).

Histology. Fixed tissues were embedded in paraffin, sectioned at 5 μm, adhered to poly-D-lysine-coated slides, and stained with hematoxylin and eosin. In situ hybridization and immunohistochemistry were performed on adjacent sections.

Probe preparation and in situ hybridization. The MVM probe was prepared as described previously (5). Briefly, the entire MVMi genome, cloned into pSP65 (Promega, Madison, Wis.), was excised from the *Bam*HI site, gel purified from 0.7% low-melting-temperature agarose (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), and labeled with ^{32}P by using a random primer extension kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). The methods of section preparation, hybridization, washes, and development have been described previously (5).

Immunohistochemistry. Rabbit antiserum to MVM capsid antigens was prepared as described previously (24). Sections were stained by the indirect streptavidin-biotin complex method used previously (5).

Semiquantitative analysis of in situ hybridization. Hybridized tissue sections were viewed at a magnification of ×100. The number of nuclei that hybridized with the MVM probe were counted for each field and each organ. The mean number of positive cells per field per organ was calculated for individual mice. A score of 0 to 4+ was assigned for each organ by dividing the total of means by the number of mice. A score of 0 indicated that no positive cells were detected in a specific organ in any mice infected with a virus genotype. A score of 1+ indicated that more than zero but less than one cell per field was positive. A score of 2+ indicated that from one to less than five cells per field were positive. A score of 3+ indicated that from 5 to less than 10 cells per field were positive. A score of 4+ signified that 10 or more cells per field were positive.

Estimation of total MVM DNA in organs. Total DNA was extracted and purified from organs by the method of Krieg et al. (14). DNA was denatured with 0.5 N sodium hydroxide, incubated at 65°C for 10 min, rapidly cooled on ice, and neutralized with ammonium acetate. Ten micrograms of denatured DNA was blotted onto nylon membranes (GeneScreen Plus; Dupont, NEN Research Products, Boston, Mass.) by using a dot-blot apparatus and baked at 80°C for 2 h. Included on the membranes were doubling dilutions of MVMi DNA cut from the *Bam*HI site of the pSP65 plasmid and gel purified. Membranes were prehybridized and hybridized with the ^{32}P-labeled MVM probe containing 5×10^6 cpm/ml as previously described (5). Membranes were autoradiographed, and amounts of viral DNA were estimated to the nearest standard dilution.

Experimental design. Clinical responses to oronasal inoculations with MVMp, MVM(1035), and MVMi(NS2-1990) were studied with one or more litters of C3H/HeSnJ mice. Litters were examined daily, and deaths were noted. Mice that survived infection were bled at 3 weeks of age, and serum antibodies to MVM were detected by a fluorescent-antibody assay (23). Selected mice that died were necropsied to determine the cause of death. Mice from 51 litters of C3H/HeSnJ mice were infected with MVMi, MVMp, or MVM(1035) and were necropsied on postinoculation days (PID) 3, 5, 7, and 9. The number of mice sampled per interval varied with litter size and mortality. From 10 to 21 mice were sampled per virus strain per interval. With MVMi(NS2-1990), a single sampling interval was selected because of the limited quantity of infectious virus stock that was available from transfected cell cultures. The interval that was selected, PID 5, was judged to be optimal for both infectious virus and viral products in tissue based on results for the other strains of virus. Three litters of mice were infected with MVMi(NS2-1990). Tissues from half the mice were

assayed for infectious virus. Tissues from the remaining mice were processed for histology, in situ hybridization, and immunohistochemistry. Total DNA was extracted from selected tissues of five mice infected with MVMi and MVM (1035) for comparison of total viral DNA by dot-blot hybridization. Five uninfected control mice were necropsied, and tissues were processed for histology, in situ hybridization, and immunohistochemistry. The clinical responses, histology, quantitative and qualitative in situ hybridization, immunohistochemistry, and peak virus titers of the mice infected with MVMi have been previously reported (5).

RESULTS

Clinical responses. Perinatal C3H/He mice were infected oronasally with approximately 10^3 focus-forming units of MVMp, MVM(1035), or MVMi(NS2-1990). MVM(1035) was MVMp with the 820-nucleotide *Eco*RI-to-*Xba*I fragment replaced by that of MVMi. This fragment encoded overlapping capsid sequences which included the allotropic determinant. MVMi(NS2-1990) was MVMi with a transversion at nucleotide 1990 which made no detectable NS2 isoforms. All 22 mice that were infected with MVM(1035) and that were monitored clinically died (average day of death, 8.9 ± 1.3). At necropsy, selected mice had bilateral renal papillary infarcts. There was no morbidity or mortality among 25 mice that were infected with MVMp or 20 mice that were infected with MVMi(NS2-1990). Mice that were inoculated with MVMp and MVMi(NS2-1990) developed serum antibodies to MVM.

Virus isolation. Infectious virus was recovered from the seven organs that were examined in mice that were infected with MVMi, MVMp, and MVM(1035) (Fig. 1). Infectious virus was not detected in the blood of mice infected with MVMp or with MVM(1035). A low-level viremia, which plateaued between PID 5 and 7, was detected in mice infected with MVMi. No infectious virus was detected in any organs of mice infected with MVMi(NS2-1990).

MVMp titers were lower than those of MVMi in the brain, liver, and spleen; similar to those of MVMi in the heart, lung, and kidney; and higher than those of MVMi in the intestine (Fig. 1). In the intestine, the peak MVMp titer was 100-fold higher than the peak titer for MVMi. MVM(1035) titers were higher than MVMi titers in all organs except the blood. The largest difference in infectivity titers (100-fold) between MVM(1035) and MVMi occurred in the heart, kidneys, and intestine. Intestinal titers of MVM(1035) were similar to those of MVMp.

Histology. The histological appearance of organs of mice infected with MVMp and MVMi(NS2-1990) was indistinguishable from that of organs of control mice. Mice infected with MVM(1035) had bilateral renal papillary infarcts after PID 5 (Fig. 2). These were identical in appearance and temporal onset to those previously described in mice infected with MVMi (5).

In situ hybridization and immunohistochemistry. Tissue sections from infected and control mice were hybridized with a double-stranded DNA probe that was derived from the entire MVMi genome. Adjacent sections were stained for the presence of MVM capsid antigens. Tissues from control mice had no detectable MVM nucleotide sequences or capsid antigens.

Semiquantitative analysis of the density of cells with nuclei that bound the MVM probe on PID 5 was performed on the eight organs listed in Table 1. The intestine of mice infected with MVMp contained more labeled cells than other

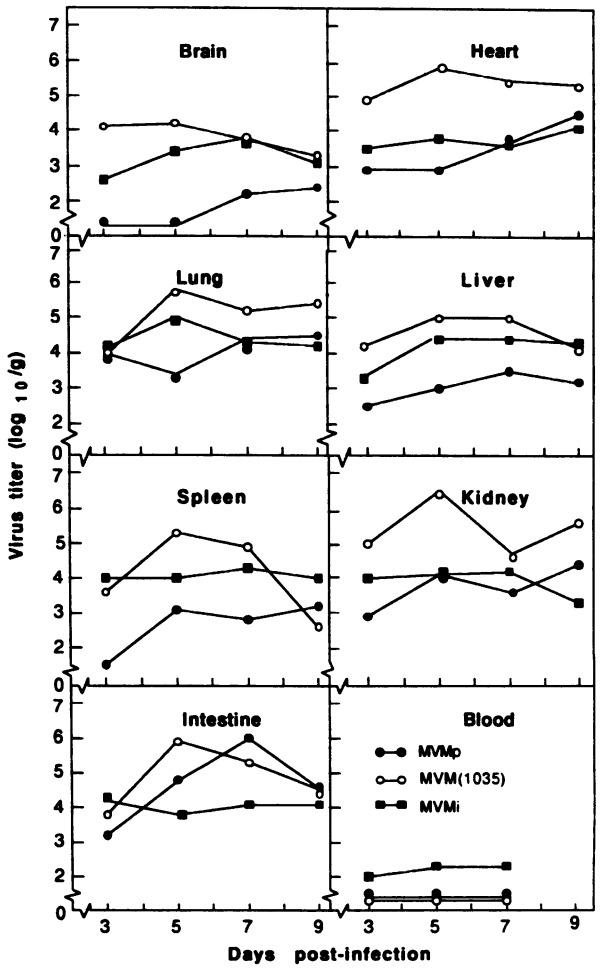

FIG. 1. Infectious virus titers in mice infected with MVMi, MVMp, and MVM(1035).

organs. The number of labeled cells in the intestine of mice infected with MVMp exceeded the number of labeled cells in the intestine of mice infected with MVMi. Organs other than intestine and lymph nodes of mice infected with MVMp had means of fewer than one labeled cell per ×100 field. Mesenteric lymph nodes contained two to five labeled cells per field, whereas other lymph nodes contained few or no labeled cells. Most organs of mice infected with MVMi had at least six labeled cells per field. The density of cell labeling was similar in organs of mice infected with MVM(1035) and MVMi. MVMi(NS2-1990) DNA was localized to the nuclei of only a few cells in the intestine, spleen, liver, and heart. A comparison of cell labeling densities in the livers of mice infected with the four MVM genotypes is shown in Fig. 3.

Cells in the lamina propria and submucosa of the small intestine, cecum, and colon of mice infected with MVMp had nuclei that were labeled by the MVM probe (Fig. 4). Similar cells in adjacent sections also expressed MVM capsid antigens. Cells that hybridized with the MVM probe in the intestine appeared to be fibroblasts based on their location and morphology. In the liver and lymph nodes, however, the few cells that were labeled did not appear to be fibroblasts. The rare labeled cells in the liver were in sinusoids either alone or among hematopoietic cells (Fig. 5). Labeled cells were scattered throughout the mesenteric

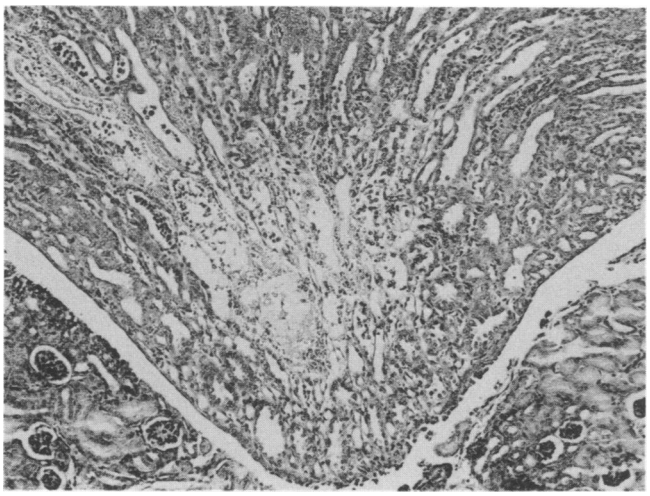

FIG. 2. Central infarct of renal papilla on PID 7 in C3H mouse infected with MVM(1035). Hematoxylin and eosin. ×160.

lymph node (Fig. 6). In no organs of mice infected with MVMp was there hybridization of the probe with endothelial cells, major sites of MVMi replication (5).

Cell nuclei that hybridized with the MVM probe in mice infected with MVM(1035) were similar in distribution and morphology to cells labeled in MVMi-infected mice (5), with several exceptions. Like MVMi, MVM(1035) replicated primarily in the capillary endothelium of the heart and kidneys (Fig. 7) and in hematopoietic cells in the liver. Nuclei of these target cells bound the MVM probe and expressed viral capsid antigens. Lymphoid cells in lymph nodes were also labeled and expressed capsid antigens.

In the brain and gastrointestinal tract of mice infected with MVM(1035), cells were labeled by the MVM probe that were not labeled in mice that were infected with MVMi or MVMp. Endothelial cells were the principal sites of MVM(1035) replication in the brain as they were in MVMi-infected mice, but in addition, cells in the external germinal cell layer of the cerebellum also contained viral DNA. The latter did not support detectable replication of MVMp or MVMi (5). In the gastrointestinal tract, mice infected with MVM(1035) had extensive labeling of smooth muscle nuclei, especially in the stomach and colon, in addition to endothelium and interstitial cells of the lamina propria and submucosa. Smooth muscle cells were not targets of infection by MVMp or MVMi (5).

TABLE 1. Semiquantitative analysis of in situ hybridization for MVM DNA in organs from neonatal C3H/He mice infected with MVMi, MVMp, MVM(1035), and MVMi(NS2-1990)

Organ	Density of nuclei that hybridized with MVM probe[a]			
	MVMi	MVMp	MVM(1035)	MVMi(NS2-1990)
Brain	+	−	++	−
Heart	+++	+	++	+
Lung	++++	+	+++	−
Liver	++++	+	+++	+
Spleen	+++	+	+++	+
Kidney	+++	+	++++	−
Intestine	+++	++++	++++	+
Lymph node	+++	++	++	−

[a] Number of positive cells per ×100 field: −, 0; +, more than 0 but less than 1; ++, 1 to less than 5; +++, 5 to less than 10; ++++, more than 10.

Cells that were labeled in mice infected with MVMi(NS2-1990) were too infrequent to accurately assess their phenotypes. They were located in the sinusoids of the liver, in the interstitium of the heart, liver, and intestine, and in the lymphoid and hematopoietic elements of the spleen.

Estimation of viral DNA in organs of mice infected with MVMi and MVM(1035). Total DNA was extracted on PID 5 from the intestine, spleen, and kidneys of mice infected with MVMi and MVM(1035), and total MVM DNA was estimated by dot-blot hybridization. There was sevenfold more viral DNA in the intestine of mice infected with MVM(1035) than in mice infected with MVMi (85 ± 22 pg/μg versus 12 ± 8 pg/μg). The kidneys of mice infected with MVM(1035) had three times more viral DNA than the kidneys of mice infected with MVMi (65 ± 34 pg/μg versus 22 ± 16 pg/μg). The spleens of mice infected with MVM(1035) and MVMi contained similar amounts of MVM DNA (170 ± 67 pg/μg and 150 pg/μg, respectively).

DISCUSSION

The failure of MVMp to replicate in capillary endothelium appeared to be the basis of its avirulence. MVMi, which is uniformly fatal in neonatal C3H/He mice, infects several cell types, but the lethal effects probably result from its tropism for capillary endothelium (5). Specifically, MVMi causes bilateral infarcts of the solitary renal papilli in association with selective virus replication in the capillary endothelium of the renal medulla. Although it has not been established that MVMi infection of medullary capillaries induces infarction or that the infarcts are the cause of death, this scenario seems likely. MVMp infected some cells in the renal medulla but fewer than were infected by MVMi, and they appeared to be fibroblasts rather than endothelium.

MVMp spread and replicated systemically, which contrasts with the findings of Kimsey and coworkers (13). They reported that MVMp remained confined to the nasal-oral cavity of perinatal BALB/c mice after intranasal inoculation, as judged by whole-body in situ hybridization, and found no evidence of virus replication. These contrasting results may have been due to differences in virus passage histories and mouse strains or in the relative sensitivities of the detection methods employed. The presence of intranuclear MVM DNA and capsid antigens in multiple organs and infectious virus in these organs at titers above the level of viremia were proof that MVMp did spread and replicate systemically.

Organ-specific infectivity titers of MVMp and MVMi were not well correlated with the density of cells in those organs with nuclei that bound the MVM probe. In organs which were equally permissive for infectious MVMi and MVMp, such as the heart, lungs, and kidneys, there were many more labeled cells in MVMi-infected than in MVMp-infected mice. We previously encountered a similar lack of correlation between infectivity titers and the number of cells labeled by in situ hybridization in neonatal C3H and C57BL/6 mice infected with MVMi (5). In that system, there is parity of organ-specific infectivity titers between mouse strains, but C3H mice have far more labeled cells than C57BL/6 mice by in situ hybridization. We have evidence that the lack of correlation in that system stems from host-mediated differences in the proportion of total viral DNA that is present in infectious virions (12a). A similar mechanism driven by differences in the viral rather than host genome could explain the disparity in this study.

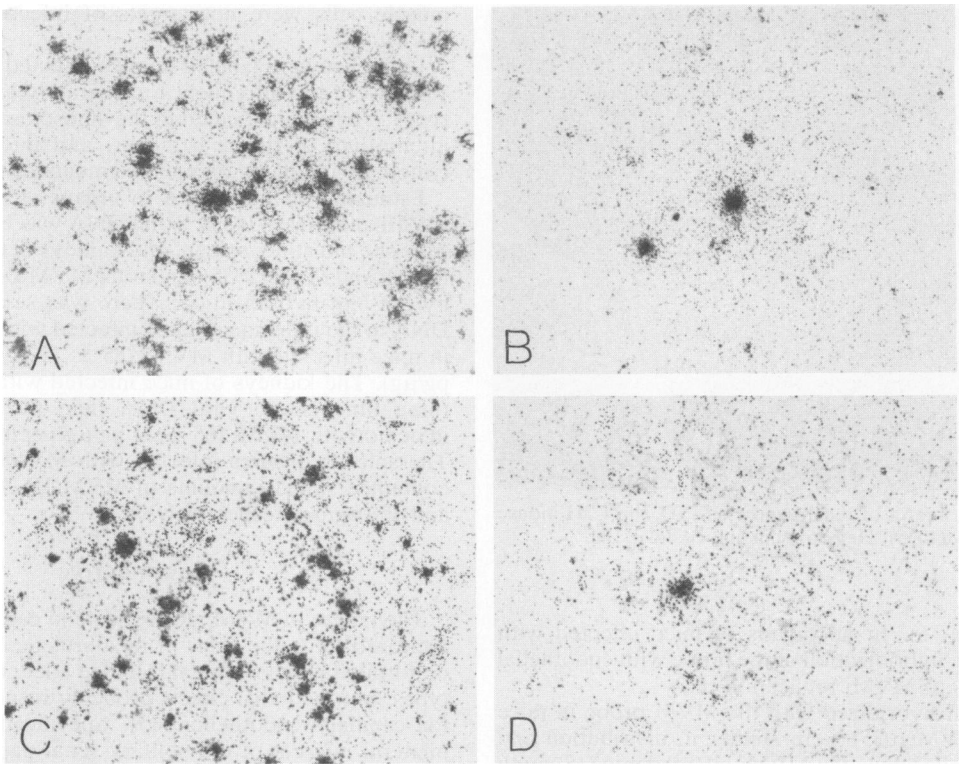

FIG. 3. In situ hybridization on PID 5 of representative liver sections of mice infected with MVMi (A), MVMp (B), MVM(1035) (C), and MVMi(NS2-1990) (D). Reversed images of dark-field illumination. ×400.

MVMp does not have tropisms for lymphocytes (24, 26) or hematopoietic cells (22) in vitro, but infected cells were common in mesenteric lymph nodes and uncommon in hepatic hematopoietic foci in vivo. There were fewer virus-positive cells in mesenteric lymph nodes of MVMp-infected mice than in MVMi-infected mice, but the distribution and pattern of nuclear labeling by the MVM probe and of nuclear staining for capsid antigens were indistinguishable. The possibility was considered that the presence of MVMp DNA and capsid antigens in lymph nodes represented sequestration of viral products originating from sites of replication in the intestine. The nuclear pattern of labeling and staining, however, suggested that this was replicating virus rather than sequestration, which would have been cytoplasmic. Alternatively, virus may have replicated in stromal cells of the lymph nodes, although morphologically, the cells appeared to be lymphocytes. One further possibility and one that is supported by the apparent limited presence of MVMp

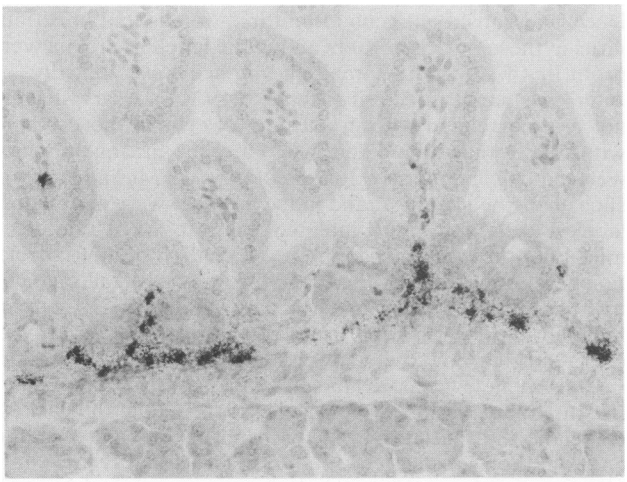

FIG. 4. In situ hybridization on PID 5 of small intestine of mouse infected with MVMp. Labeled cells are confined to the lamina propria. Hematoxylin and eosin. ×400.

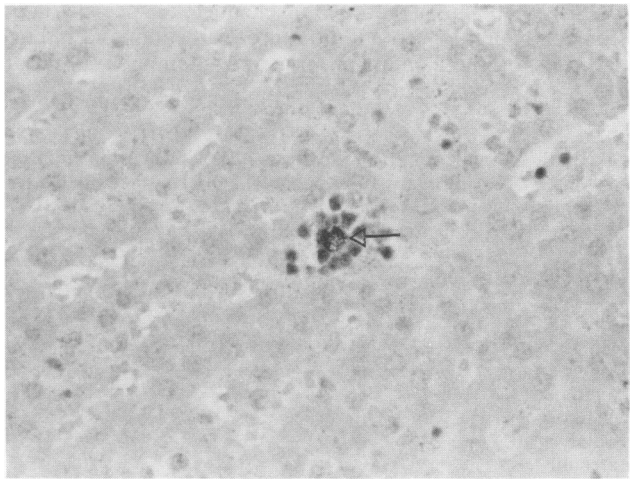

FIG. 5. In situ hybridization on PID 5 of liver of mouse infected with MVMp. A labeled cell (arrow) is among hematopoietic cells. Hematoxylin and eosin. ×650.

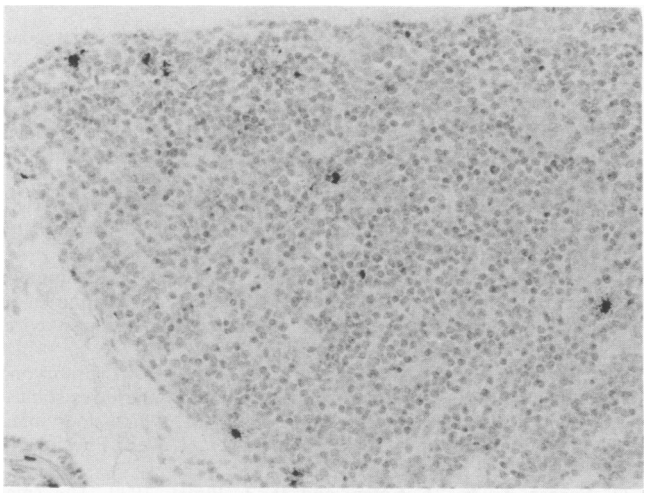

FIG. 6. In situ hybridization on PID 5 of mesenteric lymph node of mouse infected with MVMp. Labeled nuclei are scattered throughout the node. Hematoxylin and eosin. ×400.

in hepatic hematopoietic cells is that spontaneous host-range mutants developed during the limited cycles of MVMp replication in the gut which targeted cells not normally permissive for the parent virus. Future studies should examine the phenotypes and genotypes of virus that is recovered from lymph nodes and liver of MVMp-infected mice.

Phenotypically, MVM(1035) resembled MVMi. It caused a lethal infection that was associated with renal papillary infarcts, it replicated in capillary endothelium, including that of the renal medulla, and it replicated extensively in hematopoietic cells. The pathogenicity of MVMi was therefore determined by that segment of the genome between nucleotides 3522 and 4342 (820 nucleotides) which contains 35 nucleotide changes relative to MVMp. Ten of these changes alter encoded amino acids (2, 12). This region encodes overlapping sequences of VP1 and VP2 and contains the allotropic determinant (1, 12). Therefore, in addition to the lymphotropic determinant, this segment of the MVMi genome must also determine the tropisms for endothelium (5) and hematopoietic cells (5) or access of the virus to these target cells. It apparently does not determine the level of viremia, however, because MVM(1035)-infected mice did not have a detectable viremia, whereas MVMi-infected mice did.

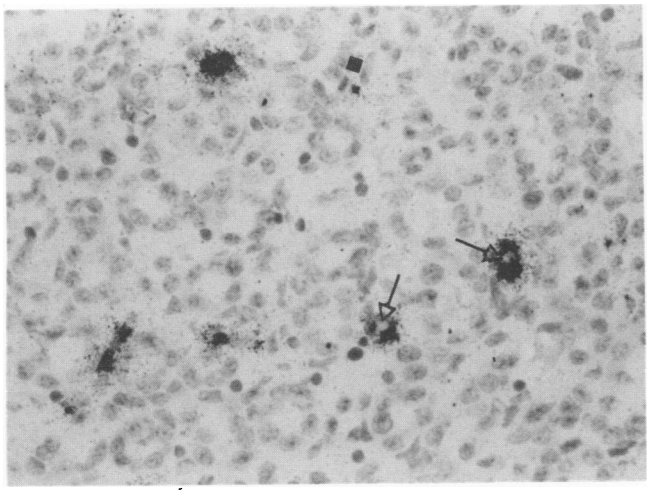

FIG. 7. In situ hybridization on PID 5 of renal medulla of mouse infected with MVM(1035). Capillary endothelial cells are labeled; several capillary lumens are shown (arrows). Hematoxylin and eosin. ×650.

Contained within the MVMp background of MVM(1035) were sequences that facilitated the synthesis of infectious virus and viral DNA relative to MVMi. This may have resulted from augmented synthesis within MVMi host cells or a broader host range. There was evidence in two organs, brain and intestine, that the host range of MVM(1035) was broader than that of MVMi. In the brain, in addition to endothelium, MVM(1035) replicated in external germinal cells of the cerebellum. We did not previously see evidence of MVMi replication in external germinal cells (5) nor were they targets of MVMp. In the intestine, smooth muscle nuclei were common sites of MVM(1035) replication. Smooth muscle cells were not detectably infected by MVMi (5) or MVMp. In organs in which no change in host range was seen, however, it seemed likely that higher titers resulted from augmented synthesis in MVMi target cells. Within the MVMp background of MVM(1035) were 128 nucleotide differences with MVMi, of which 17 encoded changes in amino acids (2). Also contained in the MVMp background was a 65-bp direct repeat between nucleotides 4720 and 4784, absent in MVMi, that contained a polyadenylation signal for all transcripts (2). This signal has been shown not to be used by MVMp, however (6).

NS2 was required for normal MVMi replication and pathogenicity. No infectious virus was recovered from organs of mice infected with MVMi(NS2-1990) despite evidence of limited spread and replication. MVMi(NS2-1990) produces less infectious virus than does wild-type virus when grown in mouse cells and is deficient in the synthesis of monomer replicative forms and progeny single strands (5a). It is not known whether the absence of detectable infectious virus was the result of detection limits, which were about 100 ID_{50}/g of tissue, or of the failure of the virus to make infectious particles despite dissemination. In either case, infectivity titers of the NS2 mutant were at least 10^2- to 10^4-fold lower than those of the parent strain in all organs, and the number of infectious centers was greatly reduced. A similar result has been reported for neonatal rats infected with H-1 virus mutated at the same R2 large splice acceptor site as MVMi(NS2-1990) and which was also unable to synthesize any NS2 isoforms (16). In that study, however, no evidence of virus spread or replication was presented, in contrast to results presented here.

The molecular bases of differences in parvoviral virulence are beginning to be examined by using the MVM-mouse model system. This system is attractive not only because so much is known about the molecular genetics of MVM but also because the genotype of the host plays an important role in MVM pathogenesis (5). Because more is known about the genome of the mouse than of any other mammalian species, it affords an opportunity to manipulate the host as well as the virus genome in order to dissect parvoviral virulence mechanisms.

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