JOURNAL OF VIROLOGY, June 1983, p. 944–955
0022-538X/83/060944-12$02.00/0

Vol. 46, No. 3

Reciprocal Productive and Restrictive Virus-Cell Interactions of Immunosuppressive and Prototype Strains of Minute Virus of Mice

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Received 26 January 1983/Accepted 24 February 1983

Viral and cellular factors responsible for parvovirus target cell specificity have been examined for two serologically indistinguishable strains of the minute virus of mice which infect mouse cells of dissimilar differentiated phenotype. Both the prototype strain and the immunosuppressive strain grow in and form plaques on monolayers of simian virus 40-transformed human fibroblasts, a finding that has allowed the comparison of several aspects of their virus-host cell interactions. Although closely related by antigenic and genomic criteria, both the prototype strain and the immunosuppressive strain are restricted for lytic growth in each other's murine host cell, that is, in T cells and fibroblasts, respectively. The host range of each virus variant appears to be specified by a genetic determinant that is stably inherited in the absence of selection. In the restrictive virus-host interaction lytic growth is limited to a small or, in some cases, undetectable subset of the host cell population, and the majority of the infected cells remain viable, continuing to grow at the normal rate without expressing viral antigens. The susceptible host cell phenotype is dominant in T lymphocyte × fibroblast fusion hybrids, implying that different cell types express different developmentally regulated virus helper functions that can only be exploited by the virus variant that carries the appropriate strain-specific determinant.

The parvovirus family consists of a large number of physically and chemically similar viruses that infect many animal species (36). These agents are small, nonenveloped, icosahedral virions approximately 20 nm in diameter, containing a 5-kilobase, single-stranded DNA genome (36). The vertebrate parvoviruses are divided into two subgroups on the basis of their requirement for helper viruses. Members of the adeno-associated virus subgroup are defective and depend entirely upon adenovirus or herpesvirus coinfection for their own replication. In contrast, members of the autonomous parvovirus subgroup are capable of productive replication without the aid of a helper virus both in vivo and in vitro.

Numerous studies over the past 20 years on the pathogenicity of autonomous parvoviruses have shown that they are predominantly teratogenic agents. In general, they cause fetal and neonatal abnormalities by destroying specific cell populations that are rapidly proliferating during the normal course of development (reviewed in references 12 and 19). These same tissues are generally resistant in the mature animal; consequently, few of the viruses cause clinical disease in the adult. However, some of these tissues can be rendered susceptible to virus infection by inducing them to undergo an abnormal proliferative response. For example, the induction of mitotic activity in the liver by partial hepatectomy (28), by carbon tetrachloride toxicity (12), and by parasite infection (13) results in parvovirus hepatitis, where virus replication is localized in the regenerating portion of the liver. A similar preferential attack on regenerating tissue is seen in parvovirus infection of healing osseous wounds (2).

Subsequent analysis of the autonomous parvovirus growth cycle in cultured cells has provided a rationale for the predilection of these viruses for dividing cells observed in vivo. These studies have shown that virus replication is dependent upon cellular functions expressed transiently during the S-phase of the cell cycle (27, 30, 33, 37). Since the viruses cannot induce resting cells to enter the S-phase (33), it is therefore not surprising that viral replication is restricted to dividing cell populations both in vitro (33) and in vivo (15). Although proliferative activity appears to be a prerequisite for target organs, it is clear that not all tissues that turn over rapidly are necessarily subject to virus-induced damage (15). Although most adult tis-

sues are mitotically quiescent compared with those of the fetus and neonate, many, such as gut epithelium and the lymphopoietic system, contain large numbers of cycling cells. One might expect these cells, which are essential for the host organism's well being and survival, to be targets for parvovirus attack in the adult. The sparing of these adult tissues by the majority of autonomous parvoviruses is underlined by the existence of a small subset of parvoviruses, namely, the feline panleukopenia-mink enteritis-canine parvovirus group and the Aleutian disease virus of mink, which frequently cause fatal disease in adult animals. The disease involves extensive destruction of gut epithelium and reticuloendothelial cells (10, 21, 26).

Studies on the replication of autonomous parvoviruses in vitro, particularly with the minute virus of mice (MVM) (38), have provided significant support for the hypothesis that lytic virus growth is modulated by developmentally regulated components operating in the host at the cellular level. Mohanty and Bachmann (23) have reported that actively dividing cells of the early mouse embryo are resistant to killing by MVM. Murine embryonal carcinoma cells, the stem cells of teratocarcinoma, are resistant to the prototype strain MVM(p), as are many of their differentiated derivatives (22, 34). However, when these cells are induced to differentiate in vitro they give rise to at least one differentiated cell type, resembling a fibroblast, which supports lytic MVM(p) replication (34). These studies suggest that cell cycling, although necessary, is not sufficient for the lytic replication of parvoviruses, and that the differentiated state of the host cell is of paramount importance.

That differences in pathogenic potential exist not only between virus serotypes, but also between virus strains of the same serotype (4–6, 11, 18, 20) suggests that a particular tissue tropism might not be an invariant property of each virus. The isolation of an additional strain of minute virus of mice, MVM(i), as an immunosuppressive agent from a murine lymphoma indicates that a mutable genetic component of the virus may play a role in determining the type of differentiated cell the virus can lytically infect. MVM(i) suppresses a number of T cell-mediated functions as measured in vitro, whereas MVM(p) does not (6), despite their genomes being closely related by restriction endonuclease mapping (20).

We describe here a single infectivity assay system for both MVM(p) and MVM(i) which has allowed us to compare directly the interactions of both virus strains with host cells of lymphocyte and fibroblast origin. The results of this study suggest that both the viral component determining target cell specificity and the developmentally regulated host factors with which it interacts can be dissected in vitro.

MATERIALS AND METHODS

Cell lines and culture conditions. A9 ouab^{r}11 cells were derived from the original HGPRT$^-$ L-cell line A9 (16) by selection for clones resistant to 10^{-3} M ouabain after nitrosoguanidine mutagenesis. A9 clone 8E is an MVM(p) resistant derivative of A9 which does not carry the receptor for MVM(p) on its surface (14). S49 1TB2 is a thymidine kinase-negative mutant of the T-cell lymphoma line S49 (3). EL4-sti is an adherent variant of the T-cell lymphoma line EL4 (8). RPC 5.4 is an immunoglobulin G2a-secreting myeloma line (24). C127 is a fibroblast line derived by Lowy et al. (17). Hyb 1/11 is a clonal hybrid line made by fusing A9 ouab^{r}11 with EL4-sti essentially by the method of O'Malley and Davidson (25) followed by selection in medium containing hypoxanthine-aminopterin-thymidine (HAT) (32) and 10^{-3} M ouabain. Hyb 2/40 is a clonal hybrid cell line resulting from a similar fusion between A9 ouab^{r}11 and S49 1TB2, followed by selection in medium containing HAT. 324K cells are a clone of simian virus 40-transformed human newborn kidney cells (29). These cells contain simian virus 40 T antigens as detected by immunofluorescent staining with monoclonal antibodies of the series generated by Harlow et al. (9), but do not produce infectious simian virus 40 either spontaneously or upon fusion with CV-1 monkey cells (unpublished results). Lymphocyte cultures were maintained in Autopow monolayer medium (Flow Laboratories, Inc.) with nonessential amino acids and 10% heat-inactivated fetal calf serum. 324K, C127, and hybrid cell lines were cultivated in the same medium containing 5% serum, and A9 lines were maintained in suspension culture in Autopow spinner medium with nonessential amino acids and 5% serum. For comparative infections all cell lines were cultured in monolayer medium with nonessential amino acids and 10% serum.

Virus stocks and infectivity assay. The original cloned stock of MVM, previously designated MVM(T), has been described elsewhere (33). This strain is now designated MVM(p)—for prototype—to avoid confusion with the preexisting nomenclature of T- and B-lymphocyte populations. Culture supernatant containing the uncloned immunosuppressive strain of MVM (4), now designated MVM(i), was generously provided by B. Hirt. This virus was cloned by terminal dilution assay in EL4 lymphocyte cultures as described below. Both viruses were assayed by plaque titration on 324K cell monolayers. Briefly, 60-mm plastic tissue culture dishes were inoculated, at 25% confluency, with 0.2-ml samples of virus diluted in monolayer medium containing 1% fetal calf serum and buffered at pH 7.3 with 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)–NaOH. After adsorption for 60 min at 37°C, the monolayers were overlaid with 8 ml of medium containing 0.6% agarose (Seakem; type ME), 0.2% tryptose phosphate, 5% serum, and buffered 15 mM HEPES–10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid adjusted to pH 7.8 with NaOH. After incubation for 6 days, plaques were visualized by staining with neutral red.

Virus production and purification. High-titer stocks of [^{3}H]thymidine-labeled virus were produced in

monolayers of 324K cells by infection at 10 PFU per cell followed by incubation in medium containing [*methyl*-^{3}H]thymidine (1 μCi/ml; 5×10^{-6} M). After 48 to 72 h, cells were scraped into the medium, collected by low-speed centrifugation, washed with phosphate-buffered saline, and finally suspended in a small volume of TE8.7 (50 mM Tris, 0.5 mM EDTA, pH 8.7) (35). The cells were lysed by three cycles of freezing and thawing, and cell debris was removed by low-speed centrifugation. After further clearing (5 min in an Eppendorf microfuge), the supernatant was layered on an 11.5-ml 10 to 30% glycerol gradient in TE8.7 and centrifuged for 2.5 h at 35,000 rpm and 5°C in an SW41 rotor. The peak of 110S infectious virions was located by fractionating the gradient from the bottom of the tube followed by liquid scintillation counting to locate the peak of labeled full virions. Peak fractions were pooled and assayed for infectivity by the 324K cell assay. All comparisons of the biological activity of the two MVM strains reported here were performed with such glycerol gradient-purified virions, unless otherwise indicated in the legend to the figure. High-titer stocks of unlabeled MVM(p) and MVM(i) were also prepared in spinner cultures of A9 ouab^{r}11 and S49 1TB2, respectively. Infected cells were harvested by centrifugation, and a TE8.7 lysate was prepared as for 324K cells. After the high-speed clearing spin, the supernatant was assayed for infectivity by the 324K assay, and dilutions were used to infect cells without further purification.

RESULTS

Isolation and assay of MVM(i). The original stock of MVM(i) was derived from the T-cell lymphoma EL4, which had been maintained as a transplantable tumor in C57/BL mice (4). The virus was isolated by growing these cells in culture until cytopathic effect (CPE) was observed, which was manifest by massive clumping of the cells followed by cessation of growth and loss of viability as measured by trypan blue staining. The uncloned stock of virus used to initiate these studies was a culture supernatant from such infected cells obtained from B. Hirt (Lausanne, Switzerland). Initial stocks were prepared by inoculating this supernatant into cultures of rapidly dividing, virus-free, EL4 cells and allowing CPE to develop. The virus was harvested from these cells by freezing and thawing in TE8.7. Such an extract was then assayed by inoculating dilutions into replicate EL4 cultures, which were then scored for CPE after each of four passages as described in Table 1. The infected cells from one of the three positive cultures at the highest dilution were extracted, and the virus was used to derive a high-titer stock in EL4 cells. Since less than 10% of the cultures at this dilution developed CPE, we assume that this stock is clonal. This assumption has been confirmed by the finding that this virus uniformly lacks an *Hin*dIII restriction site at map unit 79, which is present in approximately 80% of the genomes in the uncloned stock and uniformly present in virus subsequently grown up from another of these three CPE-positive cultures (data not shown).

TABLE 1. CPE in EL4 cultures[a]

Dilution of virus suspension	No. of wells inoculated	No. of CPE-positive wells after passage no.:			
		1	2	3	4
1×10^{-5}	20	0	20	20	20
1×10^{-6}	20	0	2	3	8
2.5×10^{-7}	40	0	2	3	3

[a] Replicate 2-ml cultures of EL4-sti inoculated with dilutions of an uncloned stock of MVM(i) were subcultured every 5 to 7 days by transferring 0.1 ml of medium plus unattached cells to 2 ml of fresh medium.

Using the initial cloned stock, we have compared the ability of this virus and the prototype strain MVM(p) to grow lytically in a number of cell lines. Although we have not found a mouse cell line that supports extensive lytic infection by both viruses, we found that two simian virus 40-transformed human newborn kidney cell lines NB-E and 324K would support both viruses and indeed afford equivalent plaque assay systems for both. Figure 1 shows that both MVM(p) and MVM(i) form plaques on 324K cell monolayers with single-hit kinetics. The low virus input required for plaque formation and the kinetics observed indicate that a single particle of each virus is capable of forming a plaque in these cells. MVM(p) is 5- to 10-fold more efficient, per particle, at forming plaques on 324K monolayers than MVM(i), and this assay for MVM(p) infectivity is about 20-fold more sensitive than the previously described assay on A9 cell monolayers (33). MVM(i), however, is at least 10^7-fold more efficient at plaque formation on 324K monolayers than on A9 monolayers.

We have used the 324K cell assay to examine the sensitivity of both viruses to neutralization by a high-titer antiserum raised in rabbits against MVM(p). Both MVM(p) and MVM(i) are neutralized with similar kinetics by this antiserum (Fig. 2). The serum appears to have a higher titer against MVM(p), which may reflect minor differences in the amino acid sequence of the capsid polypeptides between the two viruses; however, it is clear that they are antigenically very closely related. This closeness is emphasized by the fact that this antiserum has no neutralizing activity against H-1, another rodent parvovirus (Fig. 2), although H-1, MVM(p), and MVM(i) share antigenic determinants detectable by cross-immunoprecipitation of their structural polypeptides (S. F. Cotmore and P. Tattersall, manuscript in preparation).

Reciprocal susceptibility of host cells to both virus strains. The screening of murine cell lines

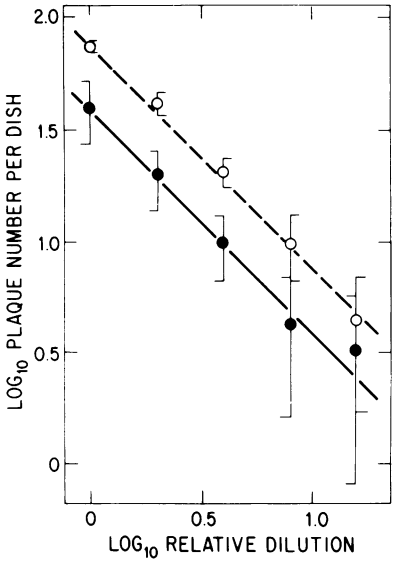

FIG. 1. Dose-response curve for MVM(p) and MVM(i) on 324K cell monolayers. Serial twofold dilutions of gradient-purified MVM(p) virions (○) and MVM(i) virions (●) were assayed for PFU with the assay described in the text. Error bars indicate the sample standard deviation for each virus dose.

for their ability to support the lytic replication of MVM(p) and MVM(i) has revealed a general pattern. Undifferentiated teratocarcinoma cells and a number of differentiated cell lines appear to be refractory to infection by both virus strains. Of the T-cell lymphomas examined, all were infected by MVM(i), but not MVM(p), and fibroblastic cell lines, including L cell derivatives, were infectable with MVM(p), but not MVM(i). On the assumption that these interactions reflect differences in the tissue tropism of the virus strains in vivo, we have examined the course of infection of both viruses in two such cell lines, the L-cell derivative A9 ouab^{r}11 and the T-cell lymphoma line S49 1TB2.

Infections of these two cell types by both MVM strains at various input multiplicities were monitored for the appearance of nuclear viral capsid antigen at 26 h after infection (Fig. 3). Since autonomous parvoviruses require a host cell function that is expressed transiently during the S phase of the cell cycle (36), the time course of appearance of viral antigen early after infection reflects the asynchrony of the cell population. However, by 26 h postinfection the majority of cells in which the input virus has established an infectious cycle are expressing high levels of capsid antigen. As can be seen, A9 and S49 cells show considerable susceptibility to infection by MVM(p) and MVM(i), respectively, as measured by this assay, throughout the range of multiplicities examined. However, the reciprocal infections show much less efficient virus takeover of the cell. Thus, in S49 cultures infected with MVM(p) all nuclei, out of the several thousand examined, were indistinguishable from those of uninfected cells, as were the great majority of nuclei in A9 cultures infected with MVM(i). Significantly, in the small fraction of antigen-positive nuclei in MVM(i)-infected A9 cultures, each nucleus appeared to express a level of viral antigen equivalent to that of positive nuclei in the reciprocal infection (Fig. 4),

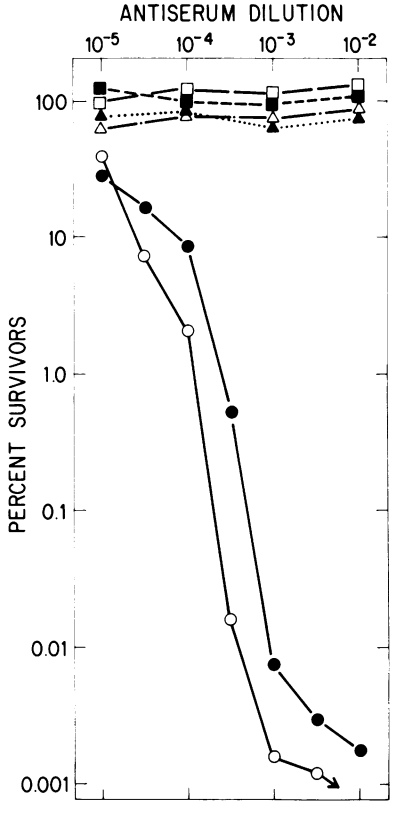

FIG. 2. Neutralization curves for MVM(p), MVM(i), and H-1 with nonimmune and anti-MVM(p) rabbit sera. A high-titer stock of each virus was mixed with dilutions of either nonimmune rabbit serum (nr serum) or a hyperimmune anti-MVM(p) serum raised in rabbits by repeated subcutaneous injection of purified MVM(p) empty capsids (αp serum). Symbols: □, MVM(p) + nr; ■, MVM(i) + nr; △, H-1 + nr; ○, MVM(p) + αp; ●, MVM(i) + αp; ▲, H-1 + αp. After incubation for 1 h at 37°C, the virus was diluted and assayed for surviving virus (PFU) as described in the text, except that the indicator monolayers were washed after adsorption to remove any residual antiserum before the overlay with agar-containing medium.

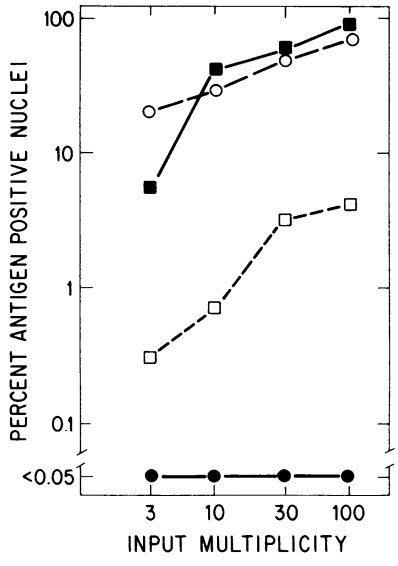

FIG. 3. Infection of A9 ouab^{r}11 and S49 1TB2 host cells with increasing multiplicities of virus. Replicate cultures of A9 ouab^{r}11 and S49 1TB2 cells at 2×10^5 cells per ml were infected at 3, 10, 30, and 100 PFU per cell with glycerol gradient-purified MVM(p) or MVM(i) virions. Cells were fixed in acetone 26 h after infection and stained by indirect fluorescence for capsid antigen. The primary antibody was the same hyperimmune rabbit antiserum raised against MVM(p) capsids described in the legend to Fig. 2, and the secondary antibody was fluorescein-conjugated goat anti-rabbit immunoglobulin. Symbols: ○, MVM(p)-infected A9 ouab^{r}11; ●, MVM(p)-infected S49 1TB2; ■, MVM(i)-infected S49 1TB2; □, MVM(i)-infected A9 ouab^{r}11.

indicating that these cells might comprise a subpopulation of cells that are lytically infectable at this input multiplicity. Thus, in the majority of each cell type, each virus showed a reciprocal difference in its ability to establish lytic infection.

Productive and restrictive virus-host cell interactions. To explore the kinetics of these virus infections and their longer-term consequences for each host cell culture, we monitored various parameters of both virus and cell growth during infection. Cultures were infected at 10 PFU per cell and followed for a time span sufficient for several cycles of virus replication, release, and reinfection. Figure 5 shows the accumulation of each virus, in terms of infectivity, in each host cell type under these conditions. In Fig. 5, the total virus produced, both extracellular and cell associated, is normalized to the number of cells originally infected. The virus titer rises to a peak value at approximately 60 h postinfection, except in the infection of S49 with MVM(p), in which no increase over early titers was detected. However, the peak titer for MVM(p) was 1,000-fold higher than that for MVM(i) in infected A9 cultures. Reciprocally, the peak titer reached by MVM(i) in S49 cells rose more than 200 times higher than the level of MVM(p) observed in a parallel infection of these cells.

When we examined the parameters of cell growth and viability, as measured by dye exclusion with trypan blue, an even more dramatic difference was found between the two types of infection. The infections of A9 with MVM(p) and of S49 with MVM(i) resulted in a rapid cessation of cell growth paralleled by a similarly rapid decline in cell viability, both starting about 40 h postinfection, at a time when exponential virus production was underway in these cells (Fig. 6). In this type of virus-host cell interaction, which we term a productive infection, the great majority of the cells in the culture are ultimately involved in virus production followed by cell death. In such host cell-virus combinations, a lowered initial multiplicity of infection merely increases the number of cycles of infection and reinfection that occur before the maximum yield of virus is achieved, which again results in the death of essentially every cell in the culture. On the other hand, the reciprocal infections of A9 with MVM(i) and S49 with MVM(p) do not appear to affect the rate of growth of the cells or their viability. Virus production and cell death appear to be restricted to a small [or, in the case of S49 cells infected with MVM(p), undetectable] subset of the whole population which, in the case of MVM(i) infected A9 cultures, appears to behave as if it was composed of normal productive host cells. However, the majority of the culture remains refractory to the infection and continues to divide without apparently being affected. We have termed this type of virus-host interaction a restrictive infection, to indicate its self-limiting nature.

Effect of multiplicity of infection on productive and restrictive interactions. We have used the assay for cell killing to investigate further the restrictive and productive interactions of both viruses with other cell lines. In these experiments, rapidly growing cells were infected at a variety of input multiplicities, and the number of trypan blue-excluding cells was determined after 5 or 6 days of growth and compared with that in control, uninfected cultures. Figure 7 summarizes the results of such assays with A9-8E, RPC 5.4, C127, and EL4-sti in addition to the A9 ouab^{r}11 and S49 1TB2 cell lines. The long-term consequences of these infections reflect the conclusions drawn above. Restrictive infections initiated at high multiplicities do not show very significant cell death, even when examined after

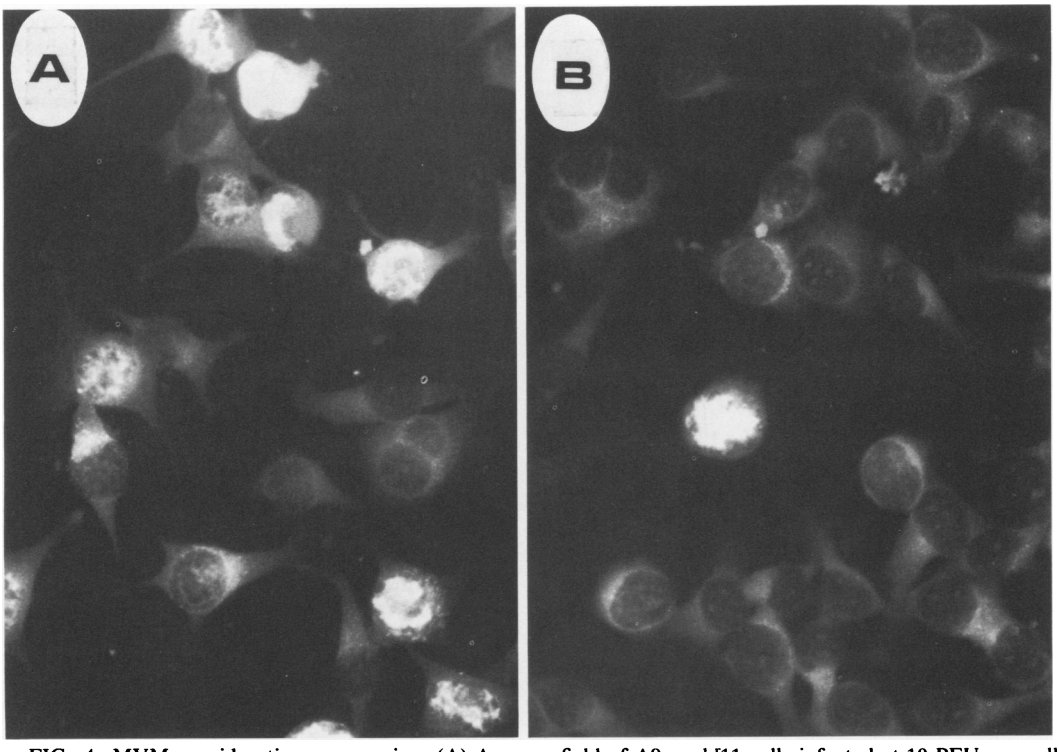

FIG. 4. MVM capsid antigen expression. (A) Average field of A9 ouab^{r}11 cells infected at 10 PFU per cell with MVM(p) as described in the legend to Fig. 3. (B) Selected field from a parallel A9 ouab^{r}11 culture infected at 10 PFU per cell with MVM(i), showing a relatively rare MVM capsid antigen-expressing cell.

time for several cycles of growth has elapsed. In the case of MVM(i) infection of A9 ouab^{r}11 at 100 PFU per cell, where almost 5% of the cells are viral antigen positive at 26 h (Fig. 3), the long-term effect on the culture appears to be little more than the removal of this small cohort of cells from the population. However, the infection of S49 1TB2 cultures with this virus at 3 PFU per cell, while inducing approximately the same fraction of infected cells in the first cycle of virus growth, led to almost 90% destruction of the culture by 5 days. It should be emphasized here that these results represent a single time point in a number of growth curves, and that this was chosen as being logistically optimal for examining the viral life cycle. Further subculture of infected restrictive host cultures does not, in general, show any change in the viability or growth rate of the culture, except in instances where a persistent infection is established, leading in some cases to the evolution of host-range mutants (D. Ron and J. Tal, personal communication; P. Tattersall, E. M. Gardiner, and J. Bratton, unpublished results). On the other hand, further subculture of infected productive host cells leads ultimately to the destruction of essentially every cell in the population, even when starting multiplicities of less than 0.001 PFU per cell are used. In all of the fibroblast lines we have examined to date, infection with MVM(p) at any multiplicity results in the eventual death of more than 99.9% of the cells and reveals a preexisting subpopulation of cells resistant to extremely high multiplicities of the virus (unpublished data).

The fibroblast line C127 and the T-cell lymphoma EL4-sti show productive and restrictive interactions with the two virus strains, similar to those shown by A9 ouab^{r}11 and S49 1TB2, respectively (Fig. 7). Interestingly, the A9 derivative A9-8E, which had been selected for the absence of the MVM(p)-specific cell surface receptor (14), and the B-cell myeloma line RPC 5.4 were completely resistant to both virus strains, at all multiplicities. As we have shown elsewhere, the resistance of these particular cell lines is due to lack of receptors for both viruses (31).

Stability of the viral phenotype. The availability of a host cell such as 324K, which supports the replication of both MVM(p) and MVM(i), has allowed us to test the stability of the strain-specific phenotype of each virus during subcloning and growth in the absence of selection.

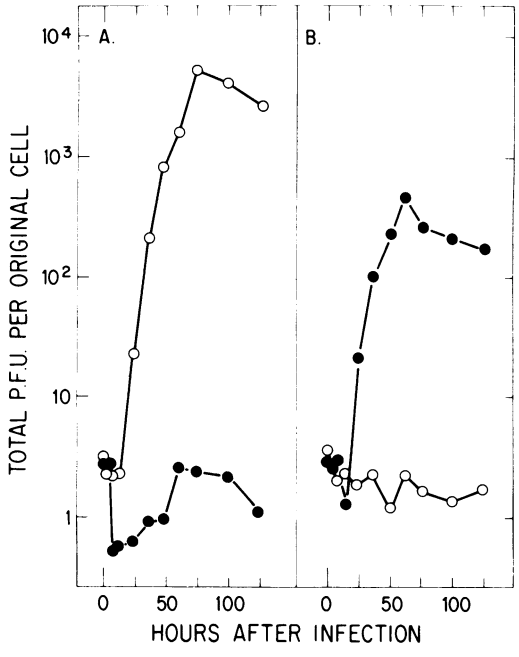

FIG. 5. Kinetics of virus production during infection. Replicate cultures of (A) A9 ouab^{r}11 and (B) S49 1TB2 cells at 2×10^5 cells per ml were infected at 10 PFU per cell with glycerol gradient-purified MVM(p) (○) or MVM(i) (●) virions. At various times after infection, samples were withdrawn and assayed for extracellular virus and cell-associated virus by the 324K cell infectivity assay as described in the text. The results are shown as the sum of these two determinations, normalized to the number of cells originally infected. Cultures were maintained between 2×10^5 and 8×10^5 cells per ml for A9 ouab^{r}11 and between 2×10^5 and 16×10^5 cells per ml for S49 1TB2 by dilution, where necessary, with fresh medium.

Single, well-isolated plaques of each virus strain were picked from 324K monolayers and grown up through two passages in 324K cultures. Titers of these stocks were then determined with the 324K plaque assay, and the cell killing ability of the stocks was measured, as a function of multiplicity, by the phenotypic assay described above. The results obtained with two independent subclones of each of the two virus strains are shown in Fig. 8. Virus stocks grown from single MVM(p) plaques maintain the MVM(p) phenotype of being able to kill A9, but not S49, cells, and MVM(i) derived subclones maintain their original, reciprocal phenotype even though they have been cloned and grown in cells that would support viruses of either phenotype. This suggests that the MVM genome carries a stable genetic determinant specifying the differentiated cell type in which the virus can grow. The stocks grown to high titer by multiple passages in 324K cells appear to be slower than the parent stocks in their kinetics of cell killing. This might be accounted for by the recent finding (M. Merchlinsky, personal communication) that high-multiplicity passage of MVM in 324K cells leads to the rapid accumulation of defective genomes such as those described by Faust and Ward (7). Two other independent subclones of each virus strain have been similarly tested and were found to give similar results (data not shown).

Restrictive host phenotype is recessive in somatic cell hybrids. To examine the nature of the block to virus replication in the majority of individuals in a restrictive host cell population, we constructed a number of A9 cell × T lymphoma hybrids and tested them for susceptibility to both viruses. The growth cycle of MVM(p) and MVM(i) in hyb 2/40, an A9 ouab^{r}11 × S49 1TB2 hybrid, initially infected at 10 PFU per cell is shown in Fig. 9. Both viruses grow in this hybrid line, reaching peak titers somewhat earlier than the infections described in Fig. 5 and 6. The peak titers per original cell of both viruses are some 20-fold lower here than in the corresponding productive infection of each of the parents of this hybrid cell line. The productive nature of the interaction of this cell line with both MVM strains is most convincingly demonstrated by the effect of each virus on cell growth and viability. (Fig. 9B and C). The more rapid kinetics of virus production, cessation of cell growth, and cell killing here compared with those observed for parental cell infections suggests that the hybrid line is more sensitive to infection at 10 PFU per cell than either of the parents. There is less than a 2-fold increase in cell number after infection of the hybrid (Fig. 9B), whereas A9 infected with MVM(p) and S49 infected with MVM(i) show a 5- and 10-fold increase, respectively, in cell number before growth ceases. When the differences in cell growth after infection are taken into account, the numbers of infectious virions produced per infected cell at the time of peak titer are comparable between two sets of infections. Taken with the growth inhibition and cell killing elicited by both viruses in hyb 2/40, we conclude that the block to infection exhibited by the majority of cells in a restrictive population behaves as a recessive trait in somatic cell hybrids.

It has not been possible as yet to demonstrate plaque formation in monolayers of hyb 2/40, probably because this cell line does not grow well under agar. However, we have examined plaque formation by both viruses in a somewhat more robust hybrid, hyb 1/11, constructed by fusing A9 ouab^{r}11 to EL4-sti. The comparison of two independently grown stocks of MVM(p) and MVM(i) for plaquing efficiency on 324K, A9 ouab^{r}11, and hyb 1/11 monolayers is summa-

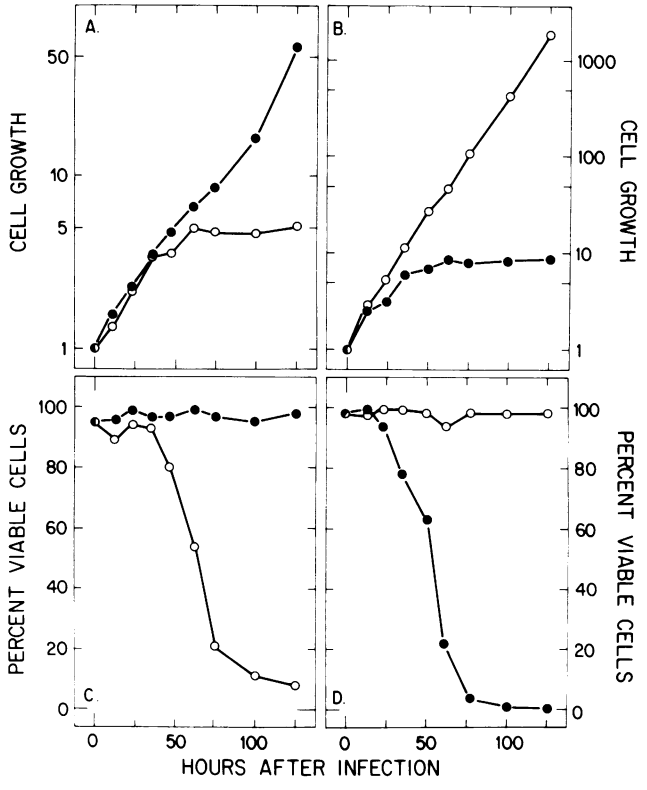

FIG. 6. Cell growth and viability during infection. Cell growth during the infections of (A) A9 ouab^{r}11 and (B) S49 1TB2 cultures with 10 PFU of MVM(p) (○) or MVM(i) (●) per cell as described in the legend to Fig. 5. Cell growth is expressed as the total cell number at each time point divided by the original number of cells present at the start of infection. Cell viability during the same infections of (C) A9 ouab^{r}11 and (D) S49 1TB2 is expressed as the percentage of cells in the culture which exclude trypan blue.

rized in Table 2. This shows that MVM(p) plaques with approximately equal efficiency on both A9 ouab^{r}11 and hyb 1/11 monolayers, although with about 1/20 of the titer demonstrable on 324K monolayers. MVM(i), on the other hand, plaques on 324K at least 10^7-fold more efficiently than on A9 ouab^{r}11; indeed, no plaques were detected in either of these assays on A9 ouab^{r}11 monolayers at the lowest MVM(i) dilutions tested. However, MVM(i) does form plaques on hyb 1/11 monolayers at about 1/100 of its efficiency on 324K cells. Given the 5- to 10-fold difference in efficiency between MVM(p) and MVM(i) plaque formation per virion on 324K monolayers mentioned above (Fig. 1), this shows that, per virion, MVM(p) and MVM(i) plaque at the same efficiency on hyb 1/11 cells. This confirms the conclusion drawn from the growth of both viruses shown in Fig. 9, that susceptibility to each virus strain is expressed codominantly in somatic cell hybrids.

DISCUSSION

In an attempt to establish an in vitro correlate for the strain-specific tissue tropisms exhibited by members of the autonomous parvovirus group (4–6, 11, 12, 15, 18–20) we have examined the virus-host interactions of two variants of MVM which infect cells of dissimilar differentiated phenotypes. The prototype strain, MVM(p), was initially isolated in whole mouse embryo fibroblast cultures and subsequently cloned by plaque purification in A9 cells (33). This virus has been the object of considerable study over the past 10 years, and many of its basic biochemical properties have been well characterized (38). The immunosuppressive strain, MVM(i), was first demonstrated as a contaminant of in vivo-passaged EL4 T-cell lymphoma cells (4), and we report here its isolation and cloning by limit dilution in EL4 cultures in vitro (Table 1). This virus grows well in a

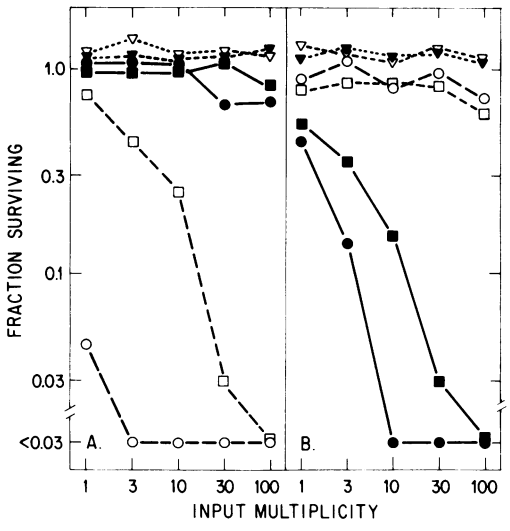

FIG. 7. Cell killing as function of input multiplicity. Replicate cultures of A9 ouab^{r}11 (○), A9-8E (▽), C127 (□), RPC 5.4 (▼), S49 1TB2 (●), and EL4-sti (■) at 10^4 per ml were infected by the addition of an equal volume of virus to give various multiplicities of infection. (A) Cells were infected with dilutions of a high-titer cleared lysate of MVM(p) grown in A9 ouab^{r}11. (B) Cells were infected with a similar preparation of MVM(i) grown in S49 1TB2. All cultures were diluted on day 2 or 3 sufficiently to keep the uninfected controls in exponential growth, and the number of trypan blue-excluding cells was determined on day 5 for RPC 5.4 and S49 1TB2 and on day 6 for the remainder. The surviving fraction is that number determined for each infected culture divided by that determined for uninfected control cultures. All points represent the averages of duplicate determinations.

number of T-cell tumor lines, especially S49 1TB2, the major host cell used in this study.

The in vitro immunosuppressive activity of MVM(i) appears to be due to its ability to kill cytotoxic T lymphocyte precursors that are responding to antigen or allogenic cells by entering the S-phase as the first step in clonal expansion (6). These responding cells do not appear to be susceptible to MVM(p) infection, although it was not possible in that study to be sure that equal infectious doses of each variant were compared (6, 20). Neither virus appears to suppress B-cell function directly (6). None of the B-cell lines we have examined to date carry specific cell surface receptors for MVM, offering a probable explanation for this finding (31; P. Tattersall, unpublished results). We show in an accompanying paper (31), however, that the susceptibility of T cells to MVM(i), but not MVM(p), is not mediated at the cell surface, but is due to a requirement for different intracellular factors for the growth of each virus.

The MVM(i) clone described here has been physically characterized and compared with the prototype strain by McMaster and colleagues (20), who found that the sedimentation coefficients, buoyant densities, and structural polypeptides of MVM(p) and MVM(i) virions appear identical. The genomes of the two variants have been compared by a number of biochemical techniques, including length measurement on alkaline gels, restriction mapping, and heteroduplex formation. The genome of MVM(i) is some 60 nucleotides shorter than that of MVM(p), and this apparent deletion has been located at approximately 92 map units, close to the 5′ end of viral DNA (20; P. Tattersall, unpublished results). So far, well over 100 restriction sites have been mapped on both genomes, and approximately 80% of them are coincident, from the left hand (3′) end in both genomes (20; E. M. Gardiner and P. Tattersall, unpublished results).

We have shown here that both of these variants will grow in, and form plaques on, monolayers of the simian virus 40-transformed human newborn kidney fibroblast cell line 324K (29). It

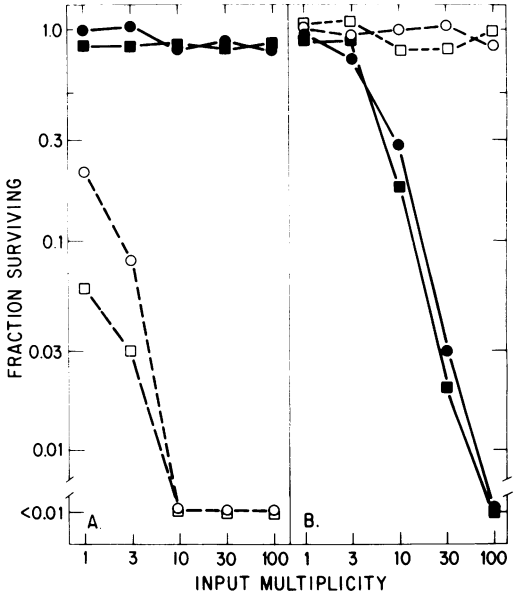

FIG. 8. Stability of virus phenotype. Independent subclones of MVM(p) and MVM(i) were isolated and expanded, titers were determined in 324K cells as described in the text, and their phenotype was determined by the cell killing assay described in the legend to Fig. 7 for (A) A9 ouab^{r}11 and (B) S49 1TB2 cells. Symbols: ○, MVM(p) subclone 1; □, MVM(p) subclone 3; ●, MVM(i) subclone 2; ■, MVM(i) subclone 3.

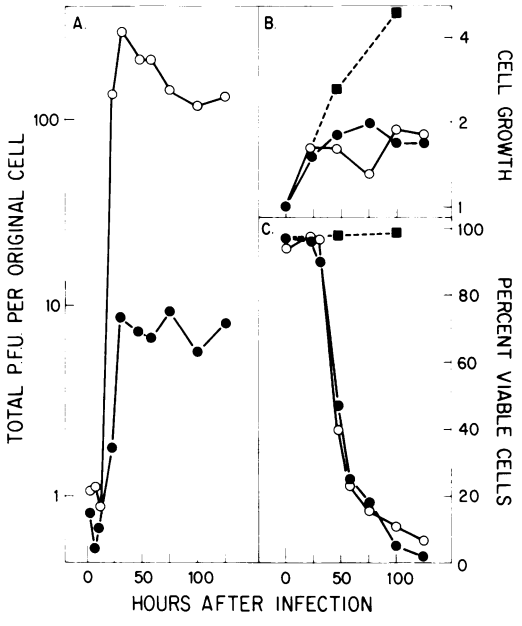

FIG. 9. Virus growth in hybrid cells. Cultures of hyb 2/40, a cloned A9 ouab^{r}11 × S49 1TB2 hybrid, were infected at 10 PFU per cell with glycerol gradient-purified virions. (A) Total PFU were determined as described in the legend to Fig. 5. (B) Cell growth and (C) cell viability were determined as described in the legend to Fig. 6. Symbols: ○, MVM(p) infection; ●, MVM(i) infection; ■, growth and viability determined for a parallel culture of mock-infected hyb 2/40 cells.

is not clear why either variant should grow at all in human cells. We have recently tested the ability of these two murine virus strains to grow in normal human fibroblast lines and have found that they both undergo abortive infection, resulting in the expression of viral antigens, but no detectable viral DNA synthesis or infectious progeny virus production (S. F. Cotmore and P. Tattersall, unpublished results). We are currently examining the role, if any, of simian virus 40 transformation in productive MVM infection of human cells.

The 324K assay has allowed us to examine several biological parameters of infection with these two MVM variants, with the assurance that we are comparing the effects of equal infectivity inputs. The two strains are serologically very closely related, perhaps indistinguishable, with heterologous antisera. Despite their high degree of physical, antigenic, and genomic relatedness, MVM(p) and MVM(i) are reciprocally restricted in their ability to grow in cell lines of T lymphocyte and fibroblast origin, respectively. We suggest the term allotropic variant to describe virus strains of the same serotype which exhibit such reciprocal interactions with host cells of the same species, but of dissimilar differentiated types. This viral phenotype is a stable characteristic of each strain even when plaque purified and amplified in 324K cells, which are permissive for either allotropic variant. We have shown that these phenotypes are also stable through plaque purification and amplification in hyb 1/11 cells, indicating that the target cell specificity of each variant is not dependent upon the phenotype of the cell in which it is grown, and therefore is unlikely to resemble the host-controlled restriction and modification systems of bacteriophages and their hosts (1). We take these results as demonstrating that the two virus variants each carry a different genetic determinant, which we call the allotropic determinant, which specifies the productive host cell type for that virus. In support of this hypothesis, we have been able to isolate, at low frequency, stable mutants of each variant which have an extended host range and can infect cells of both lymphocyte and fibroblast origin (P. Tattersall and E. M. Gardiner, unpublished results).

The existence of these two allotropic variants obviously poses the question of their origin. Do they exist in nature as separate field strains or as complex field strains comprising several distinct allotropic variants? Perhaps they arise from pantropic field strains by mutation followed by selection in the laboratory, either by passage in differentiated tumors in the whole animal or by direct isolation in cultures of differentiated cells. The availability of less stringent host cells, such as the 324K cell line described here, should allow the isolation and study of field strains in the absence of such selection.

In this paper we have also shown that the cellular component with which the allotropic determinant of the virus acts behaves in a domi-

TABLE 2. Plaque titers of virus stocks on different indicator cell lines[a]

Virus stock	Virus strain	PFU of indicator cell line per ml		
		324K	A9 ouab^{r}11	hyb 1/11
1	MVM(p)	1×10^9	5.8×10^7	1.8×10^7
2	MVM(p)	1.5×10^8	8.5×10^6	8.3×10^6
3	MVM(i)	1.7×10^8	<250	1.3×10^6
4	MVM(i)	4.7×10^8	<25	4.5×10^6

[a] Two independently grown and glycerol gradient purified stocks of each of MVM(p) and MVM(i), produced in 324K cells, were assayed for PFU on 324K, A9 ouab^{r}11, and hyb 1/11 (A9 ouab^{r}11 × EL4-sti) cell monolayers as described in the text, except that the hyb 1/11 assays were stained on day 10. Increasing the length of A9 ouab^{r}11 assay incubations does not lead to an increase in the observed titer of MVM(p) stocks or to the appearance of plaques due to MVM(i).

nant fashion in L cell × T cell hybrids. Indeed, hybrids between A9 and EL4 such as hyb 1/11 give plaques with approximately equal particle/infectivity ratios for both viruses, whereas MVM(p) is over 10^5-fold more efficient than MVM(i) in producing plaques on monolayers of the A9 parent. A simple interpretation of these results is that these two dissimilar differentiated cell types express different developmentally regulated helper functions which are exploited by the respective virus variant. That this host factor is developmentally regulated, rather than a function of our selection of cell lines derived from different mouse strains, is supported by recent experiments with BALB/c 3T3 fibroblasts. These cells are syngeneic with the S49 1TB2 T-cell line used here and are restrictive for MVM(i) but productive for MVM(p). BALB/c 3T3 cells, although restrictive for MVM(i), are, however, somewhat more susceptible to MVM(i) killing at high multiplicities of infection than the fibroblast cell lines examined here, although they maintain a 10- to 100-fold greater sensitivity to MVM(p) compared with MVM(i) over a broad range of multiplicities (P. Tattersall and J. Bratton, unpublished results). This implies that there may be germ line-transmitted differences between mouse strains in susceptibility, at the cellular level, to MVM per se.

It is of considerable importance to the understanding of parvovirus tissue tropism to determine the biochemical nature of the developmentally regulated host cell factor or factors that are involved in MVM replication and the steps in the virus growth cycle at which they act. In an accompanying paper (31) we demonstrate that, unlike the majority of examples of specific tissue tropism reported for members of other virus groups, the restriction of MVM replication described here does not occur at the level of viral cell-surface receptors, but is mediated by intracellular host factors.

Three factors, therefore, appear to act at the cellular level to confer competence as a host for MVM. First, the cell must be of the correct species; second, the cell must be traversing the cell cycle; and third, the cell must have a particular differentiated phenotype. The last factor appears to be variable, and in this case the outcome of the interaction depends upon a genetic locus within the viral chromosome for which there exist, at present, two alleles.

ACKNOWLEDGMENTS

Ric Armentrout, Peter Beard, Peter Howley, Solon Rhode, and Nancy Ruddle kindly provided cell lines. We thank Susan Cotmore, Bernhard Hirt, Solon Rhode, and Barbara Spalholz for valuable discussions.

This work was supported by a Swebilius Cancer Award and by Public Health Service grant CA29303 from the National Cancer Institute.

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