JOURNAL OF VIROLOGY, Sept. 1988, p. 3438–3444
0022-538X/88/093438-07$02.00/0

Vol. 62, No. 9

Sensitization of Transformed Rat Fibroblasts to Killing by Parvovirus Minute Virus of Mice Correlates with an Increase in Viral Gene Expression

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Received 5 February 1988/Accepted 26 May 1988

Cultures of established rat fibroblasts transformed by the avian erythroblastosis virus were more susceptible to the cytopathic effect of the autonomous parvovirus minute virus of mice, prototype strain (MVMp), than were their untransformed homologs. This effect could be ascribed to the presence of a greater fraction of cells that were sensitive to the killing action of MVMp in transformed cultures than in their normal parents. Yet, transformed and normal lines were similarly efficient in virus uptake, DNA amplification, and capsid protein synthesis. In contrast, transformants accumulated 2.5- to 3-fold greater amounts of all three major MVM mRNA species and nonstructural protein than did their normal progenitors. Thus, in this system transformation-associated sensitization of cells to MVMp appears to correlate primarily with an increase in their capacity for the expression of the viral transcription unit which encodes nonstructural proteins and is controlled by the P4 promoter. Consistently, a reporter gene was expressed at a higher level by transformed versus normal cultures, when placed under the control of the MVM P4 promoter. As infectious MVMp was produced in larger amounts by transformed cultures, a late step of the parvoviral cycle, such as synthesis, encapsidation of progeny DNA, or both, was also stimulated in the transformed cells.

Autonomously replicating parvoviruses are small, lytic, nuclear-replicating viruses which parasitize numerous animal species, including humans (1, 26). Interest in these viruses arises not only from their pathogenicity but also from the especially tight dependence on host cell functions for both their uptake and intracellular replication (6). It follows that parvoviruses can be used as probes to analyze cellular mechanisms of DNA replication and gene expression. Moreover, several cellular permissivity functions that are usurped by autonomous parvoviruses appear to be nonconstitutive; i.e., their expression is modulated by cell proliferation and differentiation (30, 33). Consequently, parvoviruses display a striking tissue specificity which is likely to be determined by cell- cycle- and developmentally regulated host proteins. In this respect, autonomous parvoviruses provide unique markers of the physiological state of host cells.

Another intriguing property of parvoviruses, which may be a reflection of the foregoing, is their ability to suppress oncogenesis that occurs spontaneously or that is induced by tumor viruses, chemical carcinogens, or transplanted tumor cells in laboratory animals (J. Rommelaere and P. Tattersall, *in* P. Tijssen, ed., *Handbook of Parvoviruses*, in press). Although the mechanism of this oncosuppression is not known, it has been speculated from the high cell requirements of parvoviruses and their frequent isolation from tumors that malignant transformation may induce normally resistant cells to become susceptible to lytic parvovirus replication (15). Such an oncolytic mechanism remains hypothetical in vivo but is consistent with results of a series of in vitro experiments. Thus, the prototype strain of minute virus of mice (MVMp), a rodent autonomous parvovirus, was shown to kill transformed derivatives of normally resistant mouse cells and, hence, to prevent the in vitro transformation of these cells (15). Moreover, the transformation of both human and rodent cells by tumor viruses, radiation, or chemical carcinogens was found to correlate with their sensitization to the killing effect of parvovirus H-1 and MVMp (4, 14).

Recent evidence suggests that the dependence of parvovirus replication on developmentally regulated cellular functions concerns multiple steps of the viral life cycle (10, 28). Therefore, the question arises as to whether cell transformation correlates with a potentiation of specific stages of parvovirus growth. A step which is limiting for the amplification of parvoviral DNA appears to be stimulated in transformed human fibroblasts compared with that in their normal parent fibroblasts (2, 3). This study was conducted to determine whether other events in the parvoviral life cycle can also be modulated as a function of cell transformation. To this end, we compared a rat cell line displaying a normal, yet immortal, phenotype with a transformed derivative induced by the avian erythroblastosis virus (AEV) for their respective susceptibilities to MVMp replication. Similar to our previous observations for other systems (4, 14, 15), the selection of an AEV-induced rat transformed line led to the isolation of a culture which was more sensitive to MVMp than was its normal parent. However, in contrast to the human fibroblast case, sensitization of the rat cells did not coincide with an increase in parvoviral DNA amplification but, rather, with a stimulation of MVMp gene expression. Thus, several steps of parvovirus replication can apparently

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be up-modulated in transformed cells compared with that in their normal progenitors.

MATERIALS AND METHODS

Cell lines and virus. A clone (208F) obtained from the established Fisher rat cell line F2408 (Rat-1) and a 208F derivative (AT1a) transformed by AEV (17, 18) were kindly provided by K. Quade (MRC, London, England). These cells were propagated as monolayers in Dulbecco modified minimum essential medium supplemented with 10% fetal bovine serum (FBS). A9, an established line of mouse fibroblasts, was maintained in Eagle minimum essential medium containing 5% FBS. Living cells were identified by the trypan blue exclusion method and counted with a hemacytometer.

MVMp was produced and purified as described by Tattersall et al. (29). Radiolabeled virus was obtained by adding [^{3}H]thymidine (80Ci/mmol; 25μCi/ml; CEA-Saclay, Saclay, France) to virus-infected cultures of A9 cells.

Titration of infectious virus and infectious centers. At intervals postinfection cultures were subjected to three freeze-thaw cycles. The suspensions were diluted in Eagle minimum essential medium containing 5% FBS, and plaques were assayed on A9 indicator cells as described previously (23). For infectious center assays, appropriate numbers of cells from virus-infected cultures were seeded on top of 4×10^5 A9 indicator cells in 60-mm-diameter dishes, which were processed for plaque assays 3 to 4 h later. The multiplicity of infection (MOI) defines the number of virus PFU inoculated per cell.

Measurement of cell clonogenicity on plastic and in semisolid medium. For the determination of cloning efficiencies on plastic, cultures were harvested by trypsinization at 4 h postinfection and replated as single cells onto 60-mm-diameter dishes. After 10 to 15 days of incubation in complete medium containing 1% anti-MVMp serum, the cells were fixed in methanol-acetic acid (3:1; vol/vol) and stained with Giemsa, and colonies were counted. Cell survival was calculated from the number of colonies formed by MVM-infected cells and expressed as a percentage of those formed by mock-treated cells. For the measurement of anchorage-independent growth, suspensions of 10^4 or 10^5 cells in 3 ml of Dulbecco modified minimum essential medium containing 10% FBS and 0.33% agar were seeded onto 60-mm-diameter dishes containing 7 ml of Dulbecco modified minimum essential medium supplemented with 10% FBS and 0.7% agar. Colonies were counted by microscopic examination between 3 and 5 weeks after they were plated.

MVMp DNA extraction and detection by hybridization. Total MVMp DNA was determined in whole-cell lysates by dispersed cell assays, essentially as described by Winocour and Keshet (32). Virus-infected cultures were harvested by trypsinization, and 5×10^5 cells were trapped by filtration onto nitrocellulose membranes. After denaturation in alkali and neutralization, the preparations were hybridized to ^{32}P-labeled, nick-translated MVM probe DNA, as reported previously (4), and counted by liquid scintillation spectrometry. The recombinant plasmid pMM984A containing the entire MVMp genome (13) was used as a probe and was a kind gift from D. C. Ward and P. Tattersall (Yale University, New Haven, Conn.).

MVMp DNA replicative intermediates were isolated from virus-infected cells (MOI, 20 PFU per cell) at various intervals postinfection by a modification of the extraction procedure described by McMaster et al. (12), and were fractionated by agarose gel electrophoresis as described by Rommelaere and Ward (24). DNA was transferred to nitrocellulose paper (27) and hybridized to ^{32}P-labeled MVMp DNA, as described above.

RNA extraction, transfer, and hybridization. Cultures were harvested 24 h after infection (MOI, 2 PFU per cell) and processed for RNA extraction. Briefly, 2×10^7 cells were incubated for 20 min at 37°C in 20 ml of STE buffer (0.1 M NaCl, 10 mM Tris hydrochloride, and 10 mM EDTA [pH 7.4]) supplemented with 0.2 g of proteinase K per ml and 1% sodium dodecyl sulfate (SDS). After phenol extraction and selective precipitation of high-molecular-weight DNA, RNA was ethanol precipitated at −70°C and collected by centrifugation in a rotor (HB-4; Ivan Sorvall, Inc., Norwalk, Conn.). Precipitates were taken up in 1.5 ml of STE buffer, and RNA was purified by centrifugation through a 1.5-ml CsCl cushion at 36,000 rpm in a rotor (SW50; Beckman Instruments, Inc., Fullerton, Calif.). Poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography and transferred onto nitrocellulose filters (Schleicher & Schuell, Inc., Keene, N.H.) either without prior fractionation (slot blots) or after denaturing agarose gel electrophoresis (Northern blots). Blots were hybridized to ^{32}P-labeled MVMp DNA (see above) and washed as suggested by the manufacturer. The autoradiograms were analyzed with a densitometer (CDS-200; Beckman). Loading of equal amounts of poly(A)-containing mRNA per lane was ascertained by slot blot hybridizations against a ^{32}P-labeled probe consisting of the rat glyceraldehyde-3-phosphate dehydrogenase gene (7).

MVMp protein immunoprecipitation and analysis by SDS-polyacrylamide gel electrophoresis. Cultures of 10^6 cells were incubated for 1 h in methionine-free medium supplemented with 0.2 mCi of [^{35}S]methionine (800Ci/mMol; Amersham Corp., Arlington Heights, Ill.). Cells were lysed, and MVMp proteins were immunoprecipitated with appropriate antisera and processed for SDS-polyacrylamide gel electrophoresis by the method of Martin et al. (11). Structural viral proteins were detected with a rabbit serum raised against empty MVMp capsids. The NS-1 nonstructural protein was recognized with a monospecific rabbit serum raised against a bacterial fusion protein which contains an MVMp-specific amino acid sequence of NS-1 (5). The proteins were run in 10% SDS-polyacrylamide gels and analyzed by fluorography. Appropriate bands were cut from the gels, and their radioactivities were determined by liquid scintillation counting.

Transient expression assays. The construction of plasmids containing the chloramphenicol acetyltransferase (CAT) gene has been described elsewhere (E. Guetta, S. Mousset, C. Bertinchamps, T. Darawshi, J. Rommelaere, and J. Tal, manuscript in preparation). Briefly, the infectious MVMp plasmid pMM984 (see above), from which the fragment *Cla*I-*Hpa*II was deleted, was digested with *Hin*dIII and *Bgl*II. A *Hin*dIII-*Bgl*II fragment containing the CAT sequence was isolated from pTS1 (31) and ligated into the cleaved pMM984 plasmid. This procedure yielded a plasmid designated pBGU7 which contained the P4 promoter and genes coding for the nonstructural proteins, with the CAT gene being approximately 700 base pairs downstream of the MVMp P38 promoter. To place the CAT gene under the control of the viral P4 promoter, a *Hin*dIII-*Eco*RV fragment was deleted from pBGU7, and the residual plasmid was religated after the strands were completed with Klenow DNA polymerase, giving plasmid pBGU8. Plasmids were grown in *Escherichia coli* HB101 and purified by isopycnic CsCl gradient centrifugation.

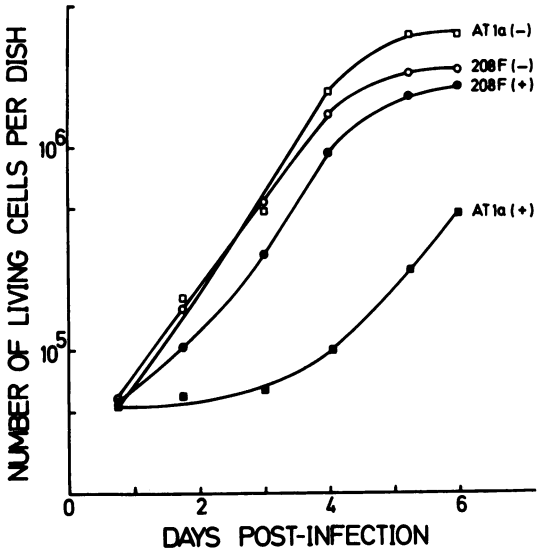

FIG. 1. Growth curves of MVMp-infected 208F and AT1a cells. Parallel cultures (5×10^4 cells) were infected with MVMp (MOI, 2 PFU per cell) or were mock treated, and the number of living cells was determined at various intervals postinfection. −, Mock-infected cells; +, MVMp-infected cells.

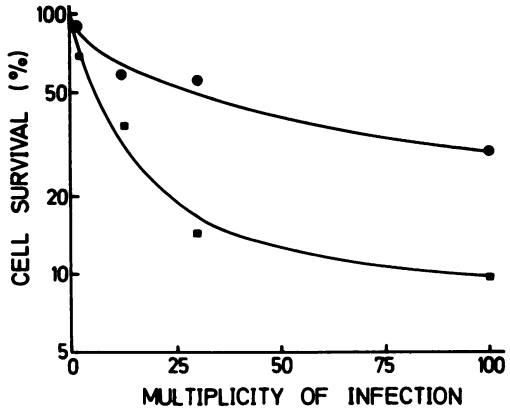

FIG. 2. Effect of MVMp infection on the colony-forming ability of 208F and AT1a cells. Cells were inoculated with MVMp, and their residual cloning efficiency was measured on plastic. The survival of MVM-infected cells was expressed as the percentage of colonies formed relative to the percentage of mock-treated cells. Plating efficiencies of uninfected cells ranged from 20 to 30%. Values are averages from two experiments (standard deviation, less than 15%). Symbols: ●, 208F cells; ■, AT1a cells.

Transfections with plasmids were performed as described by Graham and van der Eb (9). CAT assays were performed as described by Gorman et al. (8) at 40 to 48 h posttransfection, except that cell extracts were prepared by three serial freeze-thaw cycles and diluted in 0.25 M Tris hydrochloride (pH 7.8) in a final volume of 150 μl. Quantitation of CAT assays was performed by scintillation counting of the appropriate areas of the chromatography plates.

RESULTS

Effect of MVM infection on the viability of a rat cell strain and its AEV-transformed derivative. Two rat cell strains, which have been isolated and characterized by Quade and collaborators (17–19), were used in this study. Strain 208F is phenotypically normal, although it has an infinite life span. Strain AT1a was obtained from 208F by transformation with AEV and has been reported (18, 19) to contain a single copy per cell of the integrated AEV genome, which is expressed at both the transcriptional and translational level. AT1a cells can be distinguished from their 208F parent by their ability to form tumors in nude mice, rounded instead of fibroblastic appearance, higher saturation density, and much greater cloning efficiency on soft agar (18; unpublished data). Strain AT1a was also reported to be more proficient in hexose transport and to have a reduced actin cable content and a 150-fold greater plasminogen activator activity compared with 208F cells (18).

Results of initial experiments readily demonstrated that AT1a cultures were significantly more susceptible to MVMp infection than the parental 208F cell line. This was exemplified by the growth kinetics of MVM-infected 208F and AT1a cultures (Fig. 1). Samples were withdrawn at various time intervals after infection, and the number of viable cells was determined. The data indicate that in comparison with mock-infected cultures, the growth of MVMp-infected 208F cultures was slowed down moderately and that of AT1a was severely inhibited. Close to normal growth rates of the latter were achieved only after 4 to 5 days.

The delay in growth induced by MVMp may have been due to a transient viral cytostatic action on all infected cells or the existence of an MVM-sensitive fraction of cells which was killed by the virus. In the latter case, a balance would have arisen between the destruction of the MVM-sensitive subpopulation of cells and the proliferation of the rest of the culture. Such a possibility was tested by determining the residual clonogenicity of single virus-infected cells on plastic. The survival of MVM-infected cells measured as described above is plotted in Fig. 2 as a function of a wide range of input multiplicities, up to 100 PFU per cell. Cell cloning efficiency dropped with increasing virus inputs. Inactivation curves had a break point which suggested the existence of MVMp-sensitive and -resistant subpopulations of cells in both cell lines studied. Yet, the MVMp-sensitive fraction was significantly larger in the transformed cell line AT1a compared with that in its 208F parent. The enrichment of AT1a cultures in MVMp-sensitive cells may account, at least in part, for the decrease in their growth ability, compared with that of 208F cells, on infection. The resistant fraction, amounting to 30 and 9.5% of 208F and AT1a cells, respectively, survived the inoculation of MVM at high input multiplicities and readily accounted for the subsequent resumption of culture growth.

Cultures of 208F and AT1a cells grew at similar rates (Fig. 1), corresponding to generation times of about 17 h. Yet, the high dependence of parvoviruses on cell proliferation (6) raises the possibility that differences between 208F and AT1a in the duration of the various cell cycle phases might contribute to their different sensitivities to killing by MVMp. However, this possibility was not supported by the determination of the transit times of both cell lines in successive phases of the cell cycle. Strain 208F and AT1a cells could not be distinguished by the durations of G_1, S, and G_2 phases, which were estimated to be 5, 8, and 3 h, respectively, for both lines by using the labeled mitoses method. Therefore, the greater sensitivity of AT1a cultures could not be related to a major difference in the time spent in a particular phase of the cell cycle compared with that of 208F cultures. These observations do not rule out the involvement of more subtle variations in the cell cycles of the two strains.

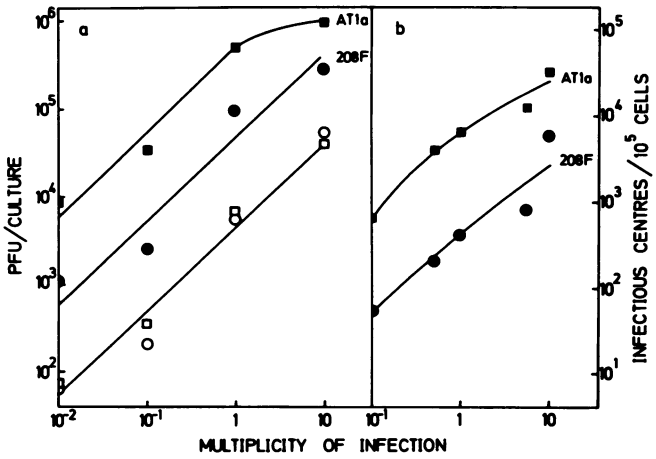

FIG. 3. Ability of 208F and AT1a cells to support a productive MVMp infection. Cultures (5×10^5 cells) were infected with increasing amounts of MVMp and processed for the measurement of virus yields (a) or infectious centers (b). (a) Titers of virus yields were determined at 2 h (open symbols) and 24 h (closed symbols) postinfection. Values are averages from two to four experiments (standard deviation, less than 30%). Symbols: circles, 208F cells; squares, AT1a cells.

Permissiveness of parental and transformed rat cells to MVMp. To characterize further the fraction of MVM-sensitive cells, parental and transformed lines were analyzed for their ability to produce infectious virus. Total virus yields, as well as the fractions of infected cells that released virus (infectious centers), were measured after inoculation with various input multiplicities of MVM. Infectious MVM was detected in both cultures (Fig. 3a), yet, the amount of progeny virus was 10-fold higher in the transformed derivative AT1a than in the parental line 208F. A similar 10-fold difference between the cultures was found in the proportion of inoculated cells that acted as infectious centers (Fig. 3b). Altogether, the data indicate that strain AT1a makes up a greater fraction of permissive cells than does the 208F parent, although productively infected cells in both cultures may release similar virus bursts. It is noteworthy that by the procedures used, the fractions of infectious centers in strains 208F and AT1a (2.5 and 23% at a MOI of 10 PFU per cell, respectively; Fig. 3b) were significantly lower than the fractions of cells killed by MVM under the same conditions of infection (30 and 64%, respectively; Fig. 2). Thus, only a fraction of killed cells was able to produce infectious centers.

Replication and expression of the MVMp genome in parental and transformed cells. The results presented above strongly suggest that the potentiation of the cytopathic effect of MVMp on AT1a cultures, compared with that on 208F cultures, is likely to result from the greater capacity of a fraction of the AT1a cells to replicate MVMp, although the viral life cycle may not necessarily go to completion. In an attempt to identify the block to MVMp growth in parental 208F cells, viral DNA replication was measured comparatively in this line and its AEV-transformed derivative. Both cultures were indistinguishable with respect to their intracellular viral DNA contents, as measured by dispersed cell assays over a range of input MOIs (Fig. 4, upper curves). The viral DNA contents of the two strains were also identical at 2 h postinfection, indicating that uptake of the inoculum was not the reason for the different permissivities of the two cell lines to MVMp (Fig. 4, lower curves). Furthermore,

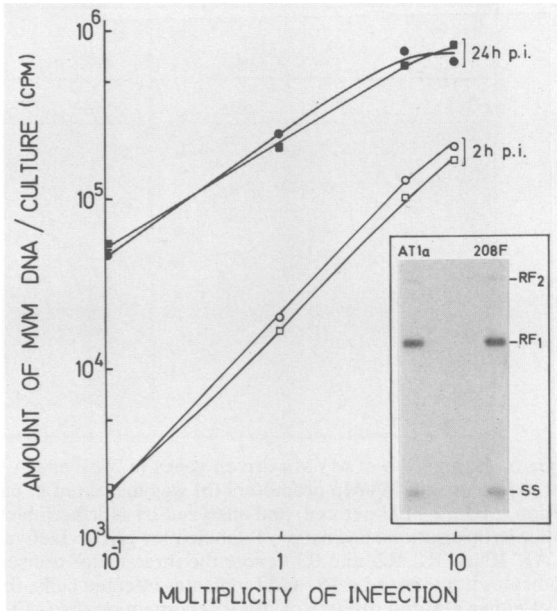

FIG. 4. MVMp DNA synthesis in 208F and AT1a cells. Cultures (5×10^5 cells) were infected with increasing amounts of MVMp. At 2 h (open symbols) and 24 h (closed symbols) postinfection (p.i.), cells were lysed and total yields of viral DNA were determined by dispersed cell assays. Values are averages from two to three experiments (standard deviation, less than 30%). Symbols: circles, 208F cells; squares, AT1a cells. (Inset) MVMp replicative intermediates extracted 24 h after virus infection (MOI, 20 PFU per cell) and analyzed by Southern blotting. SS, Single-stranded DNA; RF1, monomer-length replicative form; RF2, dimer-length replicative form.

Southern blot analysis of DNA from the supernatants described by McMaster et al. (12) did not reveal differences between the two cell lines with respect to the types and abundance of viral DNA replicative intermediates (Fig. 4, inset).

We next studied the viral RNA that was produced in 208F and AT1a cultures. Northern blot analysis of viral RNA from infected cells revealed that the three viral transcripts R1, R2, and R3 were made in both lines (Fig. 5a). However, 2.5- to 3.0-fold higher levels of all three mRNA species were present in the transformed derivative AT1a compared with the levels in the parental line 208F (Fig. 5a and Table 1). It has been reported that R1 and R2 transcripts are initiated from the same viral promoter (P4) while R3 is under the control of a separate promoter (P38) (16). The enhanced accumulation of viral mRNAs encoded by both transcription units in AT1a cultures was confirmed by transient expression assays in which a reporter gene and the procaryotic CAT gene fused to MVMp P4 or P38 promoters were monitored. For convenience, the plasmids are indicated as pP4cat and pP4NSP38cat, respectively. Thus, the former plasmid contains the CAT gene under the control of the P4 promoter, whereas the latter has the CAT gene directed by the P38 promoter in the presence of the region coding for the nonstructural proteins under the control of their own promoter (P4). Both promoters expressed higher CAT levels in transfected AT1a cultures than in 208F cells (Fig. 5b and Table 1). That DNA uptake by the procedure used was similar in both cell lines was ascertained by determination of nuclei-associated radioactivity at 30 h posttransfection with ^{32}P-labeled pMM984A DNA (data not shown).

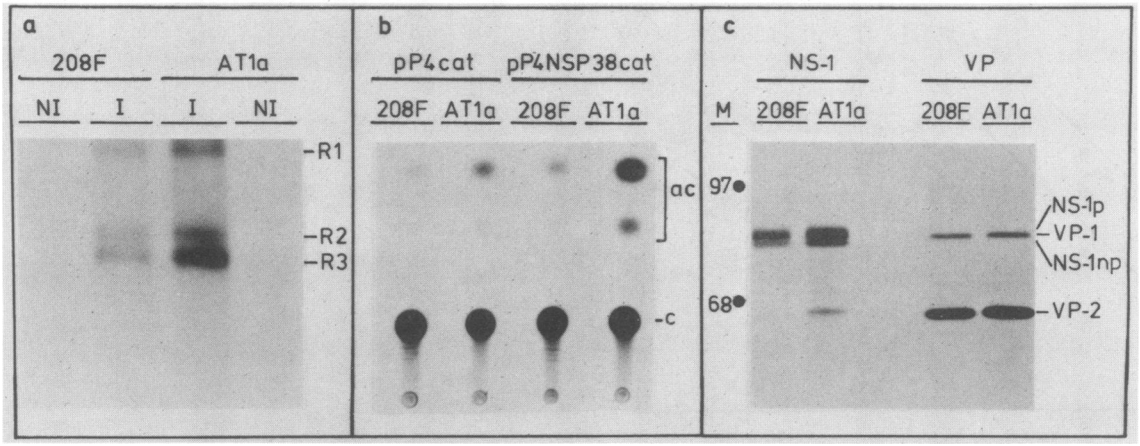

FIG. 5. Expression of MVMp-driven genes in 208F and AT1a cells. The expression of MVMp genes (a and c) or the reporter gene CAT under the control of MVMp promoters (b) was measured in parental and AEV-transformed cultures. (a) MVMp mRNA extracted 24 h after infection (MOI, 2 PFU per cell) and analyzed by Northern blotting. Amounts of poly(A)$^+$ RNA in the preparations were first determined by slot blot hybridizations against a ^{32}P-labeled rat glyceraldehyde-3-phosphate dehydrogenase gene probe. Each lane was loaded with 1 μg of poly(A)$^+$ RNA. R1, R2, and R3 denote the three major transcripts of MVM, as revealed by hybridization to a ^{32}P-labeled viral DNA probe. NI indicates noninfected cells, and I indicates infected cells. (b) Expression of the bacterial CAT gene in cultures of 10^6 cells transfected with 2 μg of either plasmid pBGU8 or pBGU7 containing the CAT gene under the control of the P4 or P38 MVMp promoter, respectively. CAT activity was determined by incubation of 50 μl of cell extract and 0.1 μCi of [^{14}C]chloramphenicol for 2 h at 37°C. Positions of nonreacted chloramphenicol (c) and two acetylated derivatives (ac) are shown. (c) SDS-polyacrylamide gel electrophoresis of radiolabeled polypeptides immunoprecipitated with specific antisera from cell extracts prepared 24 h after MVMp infection (MOI, 2 PFU per cell). Equal cell equivalents (5×10^6 cpm) of total ^{35}S-labeled proteins were used for all immunoprecipitations. M, Size markers (in kilodaltons); VP-1 and VP-2, capsid proteins; NS-1p and NS-1np, phosphorylated and nonphosphorylated forms of the NS-1 protein, respectively.

Viral structural and major nonstructural proteins were immunoprecipitated from [^{35}S]methionine-labeled extracts of MVM-infected cells by using specific antisera. The precipitates were fractionated by gel electrophoresis followed by fluorography. All three viral polypeptides that were detectable (the structural VP-1 and VP-2 capsid proteins and the nonstructural NS-1 protein) were present in both lines (Fig. 5c). Similar to other murine and human cells (5, 6; T. Dupressoir, J.-M. Vanacker, J. J. Cornelis, N. Duponchel, and J. Rommelaere, manuscript in preparation), both cultures also contained phosphorylated and poorly or nonphosphorylated forms of NS-1 (Fig. 5c), as ascertained by acid phosphatase treatment (data not shown). The transformed line AT1a, however, contained roughly 2.5 times more NS-1 than the parental 208F line (Fig. 5c and Table 1). A secondary band of approximately 65 kilodaltons was present in lysates of both infected 208F and AT1a cells that were immunoprecipitated with anti-NS-1 serum; the intensities of these 65-kilodalton bands coincided with the expression of NS-1. The nature of this protein is as yet unknown. The greater NS-1 content of the transformed derivative coincided with its enhanced capacity for accumulating the R1 transcript (Fig. 5a and Table 1) which programs the synthesis of the NS-1 protein (6). In contrast, the greater abundance of the R3 transcript in AT1a cultures (Fig. 5a and Table 1) was not accompanied by a significant increase in the amount of corresponding structural proteins (Fig. 5c and Table 1), suggesting the occurrence of a posttranscriptional limitation to the accumulation of the R3-encoded polypeptides.

TABLE 1. Gene expression of MVMp in 208F and AT1a cells

Product	Relative levels[a]
mRNAs from:	
Northern blots	2.5 ± 0.5
Slot blots	3.0 ± 0.4
Viral proteins	
VP-1	1.2 ± 0.3
VP-2	1.1 ± 0.2
NS-1	2.6 ± 0.5
CAT activity of:	
Plasmid P4cat	4.3 ± 0.3
Plasmid P4NSP38cat	9.3 ± 0.35

[a] Relative to the level in 208F cells, which was taken as 1.0. Average values ± standard deviations are from two experiments each. For experimental details, see text.

DISCUSSION

The data presented here indicate that transformation of 208F rat cells with AEV leads to the isolation of a derivative, AT1a, which is more susceptible to the cytopathic effect of MVMp than is the parent line. In this respect, the 208F-AT1a system can be added to the growing list of human and murine cell pairs for which a correlation has been found between transformation and sensitization to the autonomous parvoviruses MVM, H-1, or both (3, 4, 14, 15; Guetta et al, in preparation). Altogether, these observations raise the intriguing possibility that cell susceptibility to parvoviruses can be modulated as a function of malignant transformation. Such a modulation may account for the frequent isolation of parvoviruses from tumors (26) and may possibly contribute to the oncosuppressive activity of those viruses (Rommelaere and Tattersall, in press).

Although a fraction of AT1a cells resisted MVMp infection even at high input multiplicities, the proportion of MVMp-sensitive cells was greater in the transformed cells (AT1a) than in the parent (208F). In addition, AT1a cultures made up a greater fraction of cells that were able to support a productive MVMp infection, compared with 208F cultures. These results suggest that the increased susceptibility of the transformant to MVMp results from the greater capacity of a

fraction of the cells to sustain MVMp replication. Yet, the subpopulation of productively infected cells was significantly smaller than the fraction that was killed by the virus. This discrepancy between the numbers of sensitive and fully permissive cells indicates that a limitation to virus production takes place at a stage of the MVMp life cycle subsequent to the synthesis of viral cytotoxic factors. Similarly, certain transformed human fibroblasts are killed very efficiently by parvoviruses H-1 and MVMp, although they release little or no progeny virus (4).

On the basis of the foregoing, it was of interest to compare AT1a and 208F cells for MVMp replication. This system differed from those of the other pairs of normal and transformed cells studied so far (3, 4, 14), inasmuch as sensitization to the parvovirus was not accompanied by a detectable potentiation of viral DNA amplification. However, AT1a cultures could be distinguished from their 208F parent by their greater capacity to accumulate MVMp transcripts and NS-1 nonstructural protein. Moreover, the expression of the reporter gene CAT, which was placed under the control of either of the two MVMp promoters, was also enhanced in AT1a cultures compared with that in 208F cultures. This result, together with the similar viral DNA contents of infected AT1a and 208F cultures, strongly suggests that the increased MVMp expression that takes place in the transformant is genuine rather than a result of the greater availability of DNA templates. It therefore appears that at least two steps in the parvoviral life cycle can be stimulated in transformed cells, namely, viral DNA replication (3, 4, 14; Guetta et al., in preparation) and expression (this report; Guetta et al., in preparation).

Despite the similar abilities of both cell lines to amplify viral DNA and to synthesize capsid proteins, a higher production of infectious virus was found in AT1a cells. This apparent discrepancy remains to be worked out but raises the possibility that the assembly and/or maturation of infectious particles may be more efficient in the transformant. The latter steps of the parvoviral life cycle are dynamic processes that are associated with single-stranded DNA synthesis, modifications of capsid proteins, and concomitant cellular changes (6). It is therefore conceivable that oncogenic transformation may directly or indirectly modulate either of these events, resulting in the stimulation of infectious virus production.

The limitation to the accumulation of MVMp transcripts in a fraction of parental 208F cells is reminiscent of the block to replication of the lymphotropic strain MVMi in fibroblast lines (6; Tal, *in* P. Tijssen, ed., *Handbook of Parvoviruses*, in press). Cultures of fibroblasts, even though they were transformed and permissive to MVMp, apparently failed to express a cellular factor(s) which is required for the growth of MVMi and is present in lymphoma cells (25, 28). Such a cell- and virus-specific factor(s) has been claimed to be involved in the transcription of the MVM genome, while viral DNA replicates to a significant extent in its absence (6; Tal, in press). It is not known whether the restriction of accumulation of MVMp mRNAs in 208F cultures takes place at the level of transcription or at a later stage. Yet, based on our data and those of others, it is possible to assume that some intracellular factors which are up-modulated in transformed lines are also dependent on the cell lineage and interact with strain-specific parvoviral determinants for gene expression. Should this hypothesis prove to be correct, the correlation between transformation and sensitization to parvoviruses may be limited to some tissues and viral strains.

The genomes of the closely related autonomous parvoviruses H-1 and MVM make up two transcription units with promoters located at map positions 4 and 38, hence, their names P4 and P38, respectively (6). There is good evidence that in H-1, the nonstructural protein NS-1, a P4-controlled product, positively regulates the expression of P38-driven genes (20, 22). Identical results were recently obtained for MVM (Guetta et al., in preparation). Therefore, the primary feature distinguishing MVMp infections of AT1a and 208F cells may consist of the greater accumulation in the AT1a cell line of the R1 and R2 transcripts, which are both initiated from P4 and direct the synthesis of the viral nonstructural proteins. The enrichment of AT1a cultures with R1 RNA was accompanied by an increase in the concentration of the corresponding NS-1 protein, which might then account for the higher level of the P38-controlled R3 transcript in this cell line. The greater accumulation of NS-1 protein and R3 RNA in the AT1a cultures did not coincide with an enhancement of their content in capsid proteins, although the synthesis of these proteins is programmed by the R3 transcript. Thus, a residual limitation to the expression of capsid proteins may take place at the posttranscriptional level in AT1a cells. It should be stated, however, that this additional block could not be demonstrated in a series of other murine and human cell transformants that displayed a parallel increase in the synthesis of both NS-1 and capsid proteins, compared with those in the parental lines (Cornelis et al., in preparation). In any case, the analysis of the AT1a-208F system points to nonstructural proteins as candidates for parvoviral products which are upgraded in transformed cultures and may directly or indirectly contribute to their destruction. Results of studies that allow induction of the expression of parvoviral nonstructural genes in a cell line suggest that nonstructural proteins may indeed by cytotoxic (21). Experiments with the aim of testing the role of nonstructural proteins in the sensitization of transformed cells to parvoviruses are in progress.

ACKNOWLEDGMENTS

We thank Patrick Martin for help with some of the experiments.

This study was supported by the Commission of the European Communities and by grants from the Ministère de la Politique Scientifique, Caisse Générale d'Epargne et de Retraite, and Fonds National de la Recherche Scientifique (Belgium), and from the Institut National de la Santé et de la Recherche Médicale and Centre National de la Recherche Scientifique (France). This study was also supported by grants from the Israel Cancer Research Fund; Chief Scientist, Israel Ministry of Health; and a travel fellowship (ICRETT) from the International Union against Cancer (to J.T.). S.F.C. was supported by the Public Health Service grant CA 16038 from the National Cancer Institute.

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