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Transformation of Human Fibroblasts by Ionizing Radiation, a Chemical Carcinogen, or Simian Virus 40 Correlates with an Increase in Susceptibility to the Autonomous Parvoviruses H-1 Virus and Minute Virus of Mice

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Morphologically altered and established human fibroblasts, obtained either by ^{60}Co gamma irradiation, treatment with the carcinogen 4-nitroquinoline 1-oxide, or simian virus 40 (SV40) infection, were compared with their normal finite-life parental strains for susceptibility to the autonomous parvoviruses H-1 virus and the prototype strain of minute virus of mice (MVMp). All transformed cells suffered greater virus-induced killing than their untransformed progenitors. The cytotoxic effect of H-1 virus was more severe than that of MVMp. Moreover, the level of viral DNA replication was much (10- to 85-fold) enhanced in the transformants compared with their untransformed parent cells. Thus, in this system, cell transformation appears to correlate with an increase in both DNA amplification and cytotoxicity of the parvoviruses. However, the accumulation of parvovirus DNA in the transformants was not always accompanied by the production of infectious virus. Like in vitro-transformed fibroblasts, a fibrosarcoma-derived cell line was sensitive to the killing effect of both H-1 virus and MVMp and amplified viral DNA to high extents. The results indicate that oncogenic transformation can be included among cellular states which modulate permissiveness to parvoviruses under defined growth conditions.

Parvoviruses are small lytic single-stranded DNA viruses which rely heavily on host cell functions for their replication (41). The parvovirus family comprises three groups: the densoviruses, which infect insects, and the two more closely related groups of autonomous parvoviruses and adeno-associated viruses which have been found to parasitize birds and mammals, including man (1, 41). Helper functions for growth of adeno-associated viruses are conventionally provided by adenoviruses or members of the herpesvirus family (7). On the other hand, replication of autonomous parvoviruses (34) and, to some extent also, of adeno-associated viruses under specific stress conditions (11, 45) can take place in the absence of helper viruses in certain cell types. The cellular factors which allow the autonomous growth of parvoviruses are unknown. However, there is evidence that the expression of at least some of these factors is not constitutive but is modulated by the physiological state of cells. Function(s) restricted to the S phase of the cell cycle are necessary but not sufficient for virus replication (38, 43). In addition, cell permissiveness to parvoviruses appears to vary as a function of differentiation (37).

Interestingly, a series of transformed cells were also found to differ from their normal parents with respect to their susceptibility to parvoviruses. Thus, permanent lines of mouse and rat fibroblasts transformed by either simian virus 40 (SV40), the polyomavirus middle T gene, or the activated human Harvey *ras*-1 gene were significantly more sensitive to the lytic effect of the prototype strain of minute virus of mice (MVMp) than were their respective untransformed progenitors (19). This sensitization of transformants was accompanied by their enhanced capacity for viral DNA amplification and is likely to account for the ability of MVMp to inhibit in vitro transformation of mouse cells by SV40 (20). Parvoviruses are known to have an affinity for tumors and to antagonize oncogenesis occurring either spontaneously or upon treatment with viral or chemical carcinogens in laboratory animals (for a review, see J. Rommelaere and P. Tattersall, *in* P. Tijssen (ed.), *Handbook of Parvoviruses*, in press). The way by which the dependence of parvoviruses on conditional cellular functions contributes to such onco-suppression remains to be determined.

A choice system for studying the effect of transformation on cell permissiveness to rodent parvoviruses consists of human fibroblasts. The interaction of normal, finite-life human fibroblasts and rodent parvoviruses results in an infection which is defined as abortive (6) and is characterized by the expression of viral gene products with little viral DNA replication and without concomitant virus production. Thus, normal human fibroblasts are naturally quite resistant to the cytopathic effects of parvoviruses MVMp and H-1 virus. It was recently shown that SV40-transformed human skin fibroblasts were more permissive and sensitive to H-1 virus infection than were their normal untransformed progenitors (4). SV40 failed to provide direct helper functions for parvovirus replication (20a). However, SV40 is a strong pleiotropic transforming agent which activates cellular genes (33), raising the question whether the hypersensitivity of transformed human fibroblasts to parvoviruses is specific for SV40 transformants or is also conferred by other carcinogens. This question was addressed in the present paper by

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determining whether sensitization to MVMp or H-1 virus is a general property shared by human fibroblasts which were either transformed in vitro by ionizing radiation or a chemical carcinogen or derived from a fibrosarcoma.

Another open issue concerns the effect of cell transformation on the ongoing parvoviral life cycle. SV40 transformation of human fibroblasts was found to convert an abortive type of infection into a productive type. We were thus interested in determining whether human fibroblasts sensitized to H-1 virus or MVMp, as a result of their transformation by other oncogenes, also acquired the ability to release a burst of infectious virus. Were this not the case, it would confine events causing the death of infected cells to a window of the parvovirus life cycle. We report here that transformed human fibroblasts of different origins are all significantly more sensitive to H-1 virus and MVMp than are their respective untransformed progenitors. This sensitization parallels an increase in the permissiveness of transformants to the parvoviruses, although it does not necessarily culminate in the assembly of infectious progeny virus.

MATERIALS AND METHODS

Cell lines and culture. Finite-life human fibroblast strains of lung (MRC-5, WI38) or whole embryo (KMS-6) origin were used as untransformed control cells at passages 24 to 34 (MRC-5, WI38) or 14 to 20 (KMS-6). Transformants with infinite life spans were derived from these strains after SV40 infection (MRC-5V1) (12) or ^{60}Co gamma irradiation (WI 38CT-1, KMST-6) (23, 24). The established transformant SUSM-1 had been obtained by treatment of human embryo liver fibroblasts with 4-nitroquinoline 1-oxide (22). HT1080 is a cell line established from a human fibrosarcoma (15). MRC-5, WI38, and HT1080 have been obtained from the American Type Culture Collection via Flow Laboratories. The MRC-5V1 cells were kindly provided by A. R. Lehmann, Brighton, United Kingdom. The cells were routinely grown as monolayers in Eagle minimal essential medium supplemented with nonessential amino acids and 10% fetal calf serum. A9, a line of mouse fibroblasts, and NB-E, a line of SV40-transformed human kidney cells, were grown in Eagle minimal essential medium containing 5% fetal calf serum.

Viruses and plasmids. H-1 virus and MVMp were propagated in NB-E and A9 cells, respectively. Virus was purified according to the method of Tattersall et al. (40). ^{3}H- and ^{32}P-labeled virus was obtained by incubating infected cells with [*methyl*-^{3}H]thymidine (80 Ci/mMol; 25 μCi/ml) and carrier-free $^{32}P_i$ (0.1 mCi/ml) as described by Rhode (27) and Rommelaere and Ward (30), respectively. Conditions for cell infection and virus harvest have been described previously (5). Titers of H-1 virus and MVMp were determined by plaque assays on NB-E and A9 cells, respectively. pMM984A, a pBR322-based recombinant plasmid containing the entire genome of MVMp (17), was kindly provided by D. C. Ward, New Haven, Conn.

Virus uptake. Virus binding and uptake were determined according to the method of Linser et al. (14) with slight modifications (4). Briefly, cells were inoculated with ^{3}H-labeled virus for 60 min at 37°C. The inoculum was removed, and the cultures were further incubated for 1 h in complete medium. Cultures were then rinsed with phosphate-buffered saline deprived of Ca^{2+} and Mg^{2+} and incubated for 10 min in phosphate-buffered saline containing 10^{-3} M EDTA. The radioactivity associated with cells and that associated with pooled residual inoculum, medium, and washes were measured separately. The virus uptake was expressed as the fraction of total radioactivity which segregated with cells after EDTA treatment.

Parvovirus DNA replication. Total intracellular viral DNA synthesis was quantified either in dispersed cell lysates (44) or in blots of viral DNA isolated according to a modified Hirt procedure (16), fractionated by electrophoresis in 1% agarose gels (20 V; 18 h), and transferred to nitrocellulose filters (36). For blots, inoculated virus had tritium-labeled DNA to allow count matching of samples loaded on the gels. Viral DNA was quantitated by hybridization to pMM984A DNA labeled with [α-^{32}P]dCTP by nick translation (specific activity, 3×10^{8} cpm/μg of DNA) according to the method of Rigby et al. (29). The same probe was used to detect both H-1 virus and MVMp DNA because the genomes of these viruses display over 70% sequence homology (26). Hybridized filters of dispersed cell assays were quantitated by scintillation spectrometry, and those of blots were revealed by autoradiography at −70°C.

The conversion of parental single-stranded (SS) viral DNA to double-stranded monomeric and oligomeric replicative forms was monitored in cells inoculated with ^{32}P-labeled MVMp (30). Parentally labeled DNA was Hirt extracted and fractionated through 1% nondenaturing agarose gels as described above. Bands were localized in dried gels by autoradiography and quantitated by densitometry. Newly synthesized viral DNA was monitored by adding ^{32}P-labeled P_i to previously infected cultures in phosphate-poor medium as described by Rommelaere and Ward (30).

Virus production. H-1 virus or MVMp was inoculated to cultures (2×10^{5} cells) at multiplicities of infection (MOIs) ranging from 10^{-2} to 10^{1} PFU per cell. Cultures were further incubated in medium and frozen at intervals. The capacity of the cultures to produce infectious virus was calculated from the 2- and 120-h postinfection (p.i.) titers for the MOI of 10^{-1} PFU per cell.

Plating efficiency on plastic and cell viability. The ability of infected cells to produce colonies on plastic petri dishes was determined as described previously (19). In short, cultures (2×10^{5} cells) were infected at various MOIs. At 3 to 4 h p.i., the cells were harvested by trypsinization, plated at low densities (10^{2} to 10^{3} cells per 60-mm dish), and further incubated in complete medium for 10 to 20 days. Colonies were fixed in methanol-acetic acid (3:1, vol/vol), stained with Giemsa, and counted. The killing effect of the parvoviruses was also determined by counting the fraction of infected cells taking up trypan blue.

RESULTS

Description of cell lines. Normal and transformed human fibroblasts, including three closely related pairs, were compared for their sensitivity to the killing effect of parvoviruses MVMp and H-1 virus and for their ability to sustain viral DNA and infectious-particle production. Transformants were either induced in vitro by gamma radiation (from a ^{60}Co source), the chemical carcinogen 4-nitroquinoline 1-oxide,or a tumor virus (SV40), or derived from a fibrosarcoma (Table 1). NB-E and A9 cells, routinely used to produce H-1 virus and MVMp, respectively, were analyzed in parallel as references. Transformed human cell lines were distinguishable from their normal progenitors by an extended life span and increased plating efficiency, i.e., a greater ability to form colonies when seeded at low densities on a plastic substrate (Table 1). Moreover, transformed cells had reduced serum requirements for growth, achieved higher saturation densi-

TABLE 1. Growth properties and H-1 virus susceptibilities of human fibroblast lines[a]

Cell line	Origin	Transforming agent	Establishment[b]	Uninfected cell plating efficiency[c] (%)	Infected cell viability[d] (%)	Virus uptake[e] (%)	Amplification[f]	
							Viral DNA	Infectious virus
KMS-6	Whole embryo		−	2	75	33	16	0.3
KMST-6	Whole embryo	Gamma rays	+	15	7	38	1,330	0.2
WI38	Embryonic lung		−	1	90	18	14	0.25
WI38CT-1	Embryonic lung	Gamma rays	+	11	20	37	190	14.5
SUSM-1	Embryonic liver	4-NQO[g]	+	18	22	38	335	305
MRC-5	Embryonic lung		−	2	68	44	28	0.5
MRC-5V1	Embryonic lung	SV40	+	38	2	50	1,850	20
HT1080	Fibrosarcoma		+	22.5	5	38	635	50
NB-E	Embryonic kidney	SV40	+	60	1	35	2,160	550

[a] Average values from 2 to 3 experiments; less than 25% standard deviation.
[b] +, Established, −, nonestablished.
[c] Percentage of cells able to form colonies on plastic within 3 weeks after seeding.
[d] Percentage of living cells as measured 5 days p.i. (MOI = 10).
[e] Percentage of internalized (EDTA elution-resistant) virus.
[f] Ratio of viral DNA contents per culture at 30 h versus 2 h p.i. (MOI = 10^{-1}), as determined by dispersed cell assays, and ratio of infectious virus per culture at 120 h versus 2 h p.i. (MOI = 10^{-1}).
[g] 4-NQO, 4-Nitroquinoline 1-oxide.

ties, and displayed alterations in both their morphology and karyotypes (22–24). In contrast with normal parental cells, the transformants were capable of anchorage-independent growth, although their cloning efficiency in soft agar remained low (10^{-2} to 1%) (22–24) except for NB-E cells (5.3%). Normal fibroblasts grew at similar rates to those of their transformed derivatives (WI38 versus WI38CT-1 and KMS-6 versus KMST-6) (22–24) or even slightly faster (MRC-5 versus MRC-5V1) (12). Population doubling times ranged from 24 to 33 h.

Cell susceptibility to virus infection. The killing effect of H-1 virus and MVMp on the various cells was first determined by counting the fraction of dead cells at intervals p.i. The day 5 p.i. data are given as the most significant in Tables 1 and 2 and indicate that infection of human fibroblasts with either parvovirus results in a reduction of the fraction of living cells in the culture. Moreover, transformed fibroblasts appear to suffer this viral toxic effect to a greater extent than corresponding normal cells. This method does not allow one to quantitate viral cytopathogenicity because of the multiplication of resistant cells and the disintegration of dead cells. Therefore, virus-induced killing was also determined by measuring the residual ability of infected cells to form colonies on plastic substrate. This assay confirmed both the toxicity of H-1 virus and MVMp for normal human fibroblasts and the greater susceptibility of transformed cells to this effect (Fig. 1). The transformants MRC-5V1, KMST-6, and WI38CT-1 were 75 to 1,150 times more sensitive to the lytic action of H-1 virus than their untransformed progenitors at an MOI of 20 PFU per cell (Fig. 1a). Similarly, the chemically transformed and fibrosarcoma-derived lines SUSM-1 and HT1080 were hypersensitive to H-1 virus (Fig. 1a). MVMp also reduced the cloning efficiency of transformed fibroblasts to a greater extent than that of normal cells. Yet MVMp was less cytotoxic than H-1 virus for all human fibroblasts tested, whether normal or transformed (Fig. 1; Tables 1 and 2).

Virus uptake. Data presented in Tables 1 and 2 clearly show that the transformation-associated sensitization of human fibroblasts to H-1 and MVMp cannot merely be ascribed to their greater virus uptake compared with normal cells. No consistent relationship was observed between the amount of internalized virus and the degree of cell sensitivity. Only WI38 cells took up significantly less ^{3}H-labeled virus than the other cells. Similarly, the amounts of cell-associated viral DNA detected at 2 h p.i. by dispersed cell assays were not significantly different between untransformed and transformed cells (data not shown). Thus, cell-dependent restrictions to virus uptake, which often determine cell susceptibility to various viruses, do not appear to play a major role in the system studied.

Viral DNA synthesis. Parvovirus DNA synthesis can schematically be divided in three stages: (i) the conversion of SS viral DNA to a double-stranded monomer-length replicative form (RF1); (ii) the amplification of RF1 by a process which is accompanied with the formation of multimeric molecules such as a dimer-length replicative form (RF2); and (iii) the synthesis of SS, progeny viral DNA (6, 41).

The fraction of input parvovirus SS DNA which is converted to RF molecules is rather low but can easily be demonstrated for MVMp infecting permissive mouse A9 cells (30). One should bear in mind that MVMp parental DNA conversion may be masked by the spontaneous reannealing of input viral strands. Indeed, MVMp virion stocks comprise a small fraction (on the order of 1%) of particles containing plus strands which can reanneal with major negative strands in infected cells or their lysates, thereby setting a lower limit to the amount of converted parental RF which can be detected (3). When the fate of ^{32}P-labeled MVMp DNA was analyzed in human fibroblasts, a small fraction of input DNA was found to migrate at the RF1 position for all cells tested. However, in contrast with the situation in A9 cells, parentally labeled RF DNA did not accumulate in human cells with time p.i. and did not comprise a measurable fraction of molecules with covalently bound viral and complementary strands (data not shown). Therefore, it appears that MVMp DNA conversion in both normal and transformed human fibroblasts was too low to be distinguished from spontaneous strand reannealing, thereby preventing us from comparing both types of cells for their proficiency in this process.

However, a striking difference was observed between normal and transformed cells when total parvovirus DNA synthesis was measured by dispersed cell assays (Fig. 2; Tables 1 and 2). All tested lines had similar viral DNA contents at 2 h p.i., confirming that they do not differ significantly in their virus uptake efficiencies (data not shown). In contrast, the various cells could be distinguished

TABLE 2. Characteristics of MVMp infection of human fibroblasts[a]

Cell line	Cell viability (%)	Virus uptake (%)	Amplification	
			Viral DNA	Infectious virus
KMS-6	90	46	15	0.1
KMST-6	40	40	340	2.5
WI38	91	24	1	0.3
WI38CT-1	75	37	10	2.0
SUSM-1	77	39	25	14.5
MRC-5	85	45	2	0.15
MRC-5V1	53	43	20	18.5
HT1080	35	45	725	17.0
A9	40	38	1,750	10^4

[a] Experimental conditions and definitions are the same as for Table 1.

by the amount of viral DNA accumulated during p.i. incubation. The parvovirus DNA content of the cultures at 30 h p.i. increased with the MOI up to a plateau, giving their maximal capacity for viral DNA synthesis (Fig. 2). All transformants had a greater H-1 virus and MVMp DNA capacity than normal cells, although there was a considerable variation between lines. The extent of viral DNA synthesis was greater for H-1 virus than for MVMp in all human fibroblasts studied, with the exception of HT1080. The amplification of input parvovirus DNA achieved by cells infected at an MOI of 10^{-1} PFU per cell (within the linear part of the dose-response curve; Fig. 2) is given in Tables 1 and 2. The comparison of related pairs of cells indicates that the factor of parvovirus DNA amplification was 14- to 85-fold (H-1 virus) and 10- to 23-fold (MVMp) higher in the transformants than in their normal progenitors. As a whole, the proficiency of transformed human fibroblasts in the amplification of H-1 virus or MVMp DNA can be put together with their hypersensitivity to the lytic effect of these viruses, although no strict correlation was found between the levels of both parameters (compare Fig. 1 and 2).

To determine whether the viral DNA replicative intermediates present in fully permissive mouse A9 cells were also produced in human fibroblasts, total MVM DNA extracted from infected cultures was blot hybridized after fractionation by agarose gel electrophoresis (Fig. 3). RF DNA could hardly be detected in normal cells which essentially contained SS DNA of probable input origin (Fig. 3, lanes 3 to 4 and 7 to 8). In contrast, transformed and tumor-derived human fibroblasts sustained a clear-cut production of MVMp RF1 and RF2 intermediates (Fig. 3, lanes 5 to 6, 9 to 12 and 15 to 17). Similar results were obtained for H-1 virus-infected cells (4; data not shown). Mouse A9 cells accumulated MVMp progeny SS DNA in addition to RF molecules (Fig. 3, lanes 1 to 2). Such a MVMp progeny strand synthesis could not be demonstrated by this method in the transformed fibroblasts under study. As exemplified in Fig. 3 for HT1080 cells, total SS MVM DNA did not amplify with p.i. time and may thus consist mostly of input strands (Fig. 3, compare lanes 13 and 14 with 15 and 17).

That human cell transformants may exhibit some deficiency to accumulate SS progeny DNA despite high levels of total viral amplification was confirmed by growing MVMp or H-1 virus-infected cells in the presence of ^{32}P-labeled P_i. Transformed cultures achieved little or no detectable de novo synthesis of parvovirus SS DNA while accumulating RF molecules. This is illustrated by Fig. 4, showing that newly synthesized viral DNA present in mouse A9 and human NB-E cells consists of SS, the recently described 8.0-kilobase intermediate (9), RF1, and RF2, whereas only

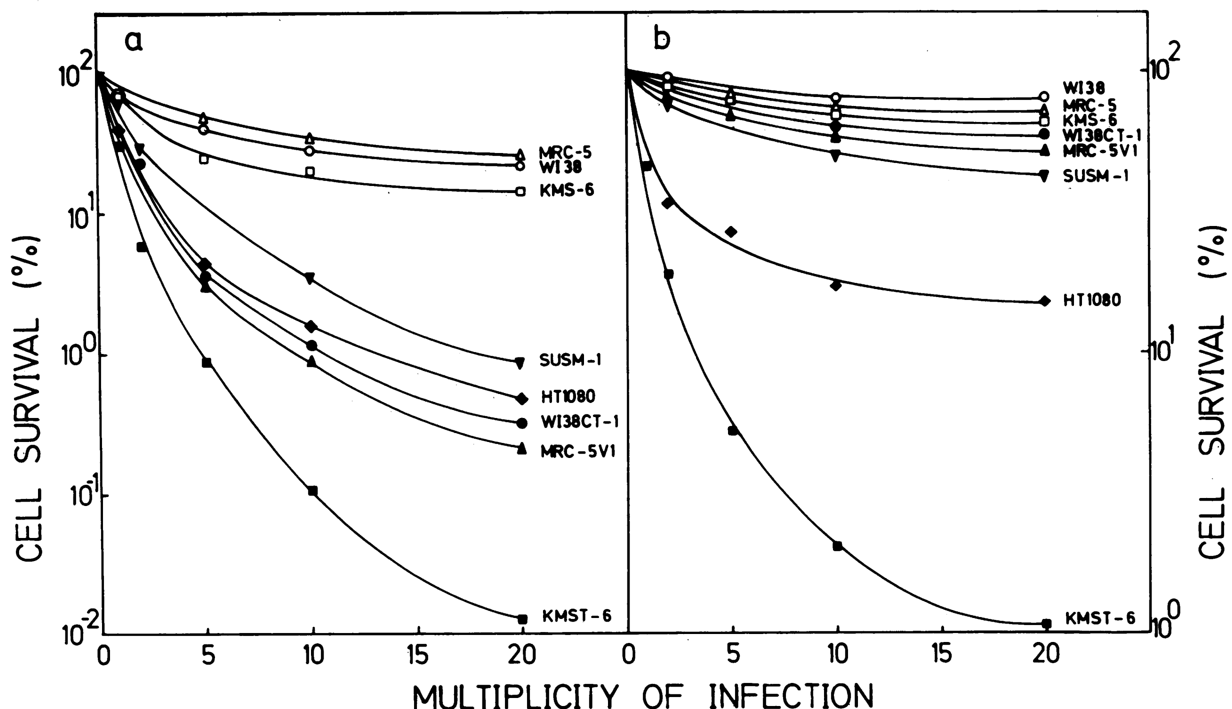

FIG. 1. Susceptibility of human fibroblasts to the killing effect of H-1 virus (panel a) or MVMp (panel b). Cells were infected at increasing multiplicities (PFU per cell), and their survival was determined by measuring their residual colony-forming ability. Open symbols, untransformed cells; closed symbols, in vitro transformed or tumor-derived cells.

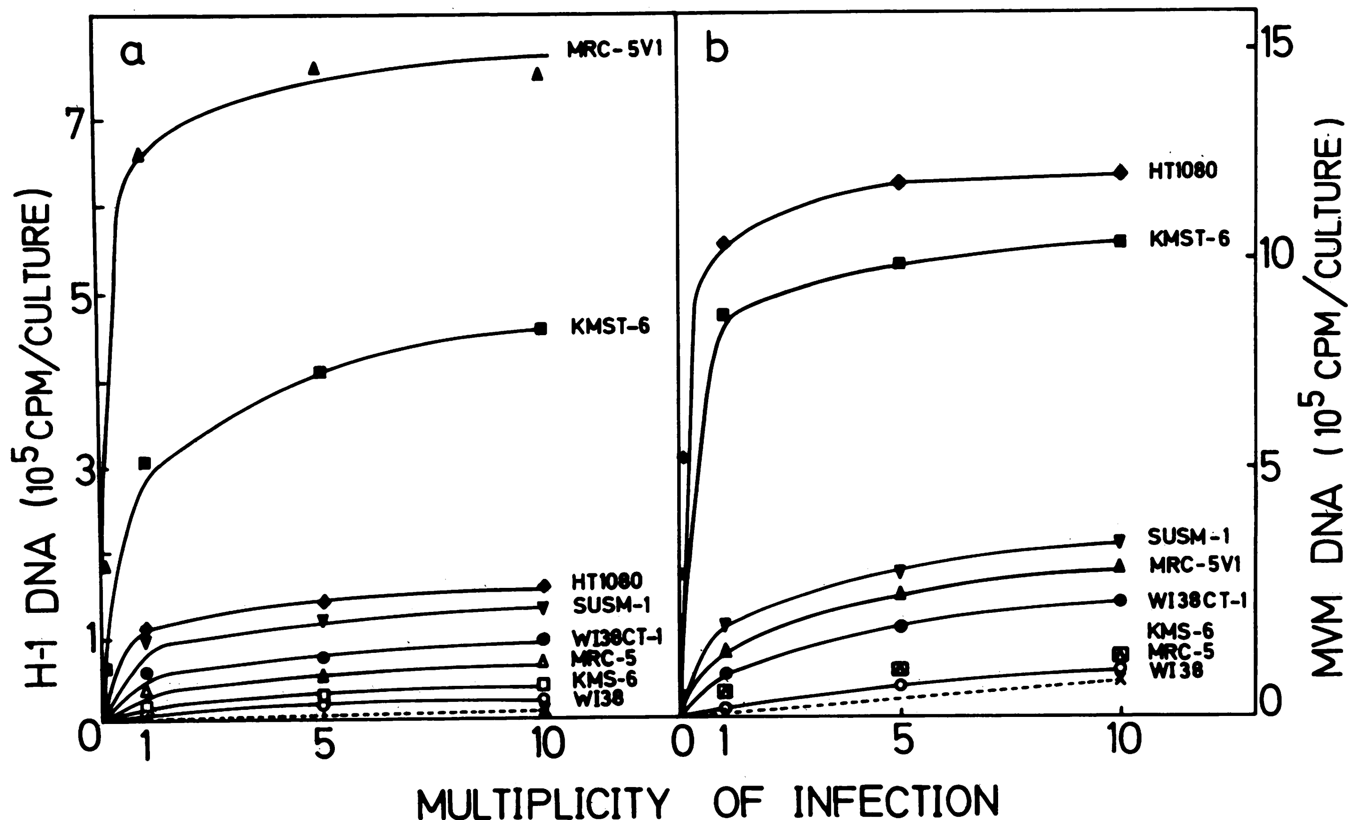

FIG. 2. Accumulation of H-1 virus (panel a) or MVMp (panel b) DNA by normal and transformed human fibroblasts. Cultures were infected at increasing multiplicities, and their viral DNA contents were determined by dispersed cell assays at 30 h p.i. The amount of input DNA, as measured 2 h p.i., was similar in all cells and is given for comparison (broken line).

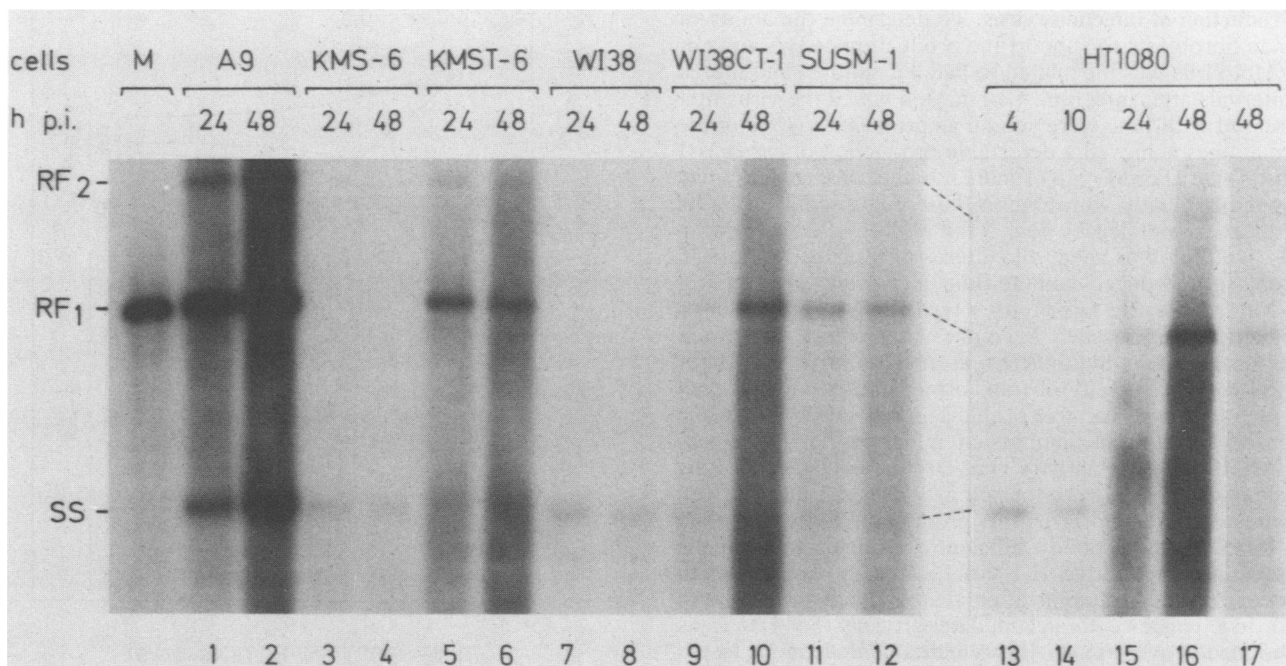

FIG. 3. Pattern of MVMp DNA present in normal and transformed human fibroblasts. Cells were infected with ^{3}H-labeled MVMp (2×10^{5} cpm per culture) at an MOI of 2 to 5 PFU per cell. Hirt extracts were prepared at intervals p.i., fractionated by gel electrophoresis, and blot hybridized against a ^{32}P-labeled probe. Samples were adjusted to the same amount of input DNA (3.6×10^{3} cpm of [^{3}H]DNA per lane) except for A9 cells (1.8×10^{3} cpm of [^{3}H]DNA). In vitro converted virion DNA was used as a marker (M). Lanes 16 and 17 represent the same blot, with a five-times-shorter exposure to film for the latter lane.

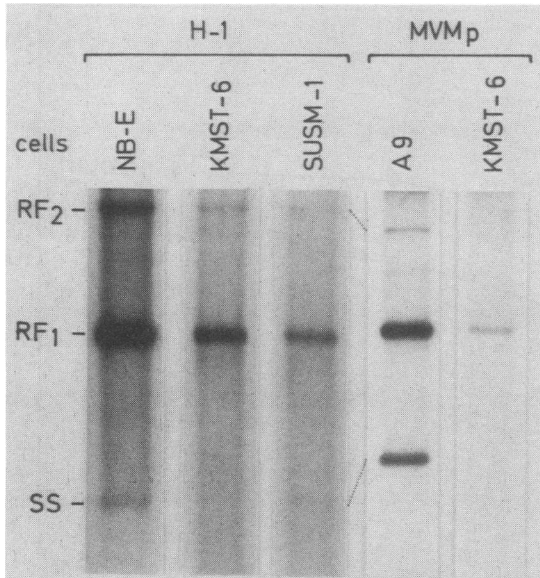

FIG. 4. MVMp DNA synthesis in sensitive human and rodent cells. A9, NB-E, KMST-6, and SUSM-1 cells were infected with H-1 virus or MVMp at an MOI of 5. $^{32}P_i$ (66 μCi/ml) was added 10 h later, and Hirt extracts were prepared at 24 h p.i. Lanes were count matched with 8×10^5 cpm of ^{32}P-labeled DNA. The autoradiograms of dried gels are shown.

the latter two forms could be found in the transformed human lines KMST-6 and SUSM-1. Therefore, transformed human fibroblasts provide cases in which the production of monomeric and oligomeric RFs contributes to parvovirus DNA amplification without concomitant formation of SS viral DNA.

Production of infectious virus. To determine the ability of human fibroblasts to support the production of H-1 virus or MVMp, virus was inoculated to parallel cultures and titered at intervals after infection. Cell lines in which the virus titer increased with time were scored as producers. None of the normal lines achieved a detectable production of either H-1 virus (Table 1) or MVMp (Table 2). Indeed, parvovirus titers dropped gradually with time in these cultures for all MOIs used, as exemplified by Fig. 5 for MVMp-infected KMS-6 cells. On the other hand, all cultures of transformed human fibroblasts produced minute but significant amounts of MVMp (Table 2). Given its very small extent, such a production was masked by input virus unless cells were infected at a low multiplicity, as illustrated in Fig. 5 for KMST-6. The capacity of transformed cultures for producing H-1 virus was variable (Table 1). Only the SUSM-1 line released appreciable amounts of infectious H-1 virus, although its ability to amplify viral DNA was moderate. Low levels of H-1 virus were formed in WI38CT-1 and HT1080 cultures, whereas no H-1 virus production was detected in the KMST-6 line. The low efficiency of most transformants to sustain a productive H-1 virus infection despite a high level of viral DNA amplification (Table 1) may be related to their low capacity to accumulate progeny SS DNA, as mentioned above (Fig. 4). It is remarkable that among tested transformants, the most H-1 virus-sensitive line, KMST-6, was not producing virus, whereas the least sensitive one, SUSM-1, was the best producer (Fig. 1a; Table 1). This lack of a correlation between sensitivity and production indicates that the acquisition of full permissiveness to the parvovirus is not a prerequisite for the sensitization of transformed human fibroblasts to its cytopathic effect.

DISCUSSION

Two main findings are reported in this paper. First, transformation of human fibroblasts correlates with an increase in their sensitivity to autonomous parvoviruses H-1 virus and MVMp, irrespective of whether transformation is induced in vitro by a tumor virus, radiation, or a chemical mutagen or whether it takes place spontaneously. This result suggests that the occurrence of step(s) of human cell transformation is associated with the expression of a parvovirus-hypersensitive phenotype. Second, the greater cytopathogenicity of parvoviruses for transformed versus normal cells parallels a higher level of viral DNA replication in the former cultures, although infection is not necessarily productive. One may conclude from the latter observation that the hypersensitivity of transformed human fibroblasts to H-1 virus and MVMp is likely to reflect an up modulation of their permissiveness to these viruses but does not require the viral life cycle to go to completion.

Low susceptibility of normal human fibroblasts to H-1 virus and MVMp. The data presented show that all three finite-life cultures of embryonic human fibroblasts tested are unable to sustain a productive H-1 virus or MVMp infection. This result confirms and extends previous observations by us and others, showing that normal human fibroblasts, whether of embryonic, neonatal, or adult origin, fail to produce infectious H-1 virus (4, 13, 35, 42). Untransformed fibroblasts undergo an abortive type of H-1 virus or MVMp infection, characterized by normal virus uptake but very limited intracellular viral DNA amplification. On no occasion could RF2

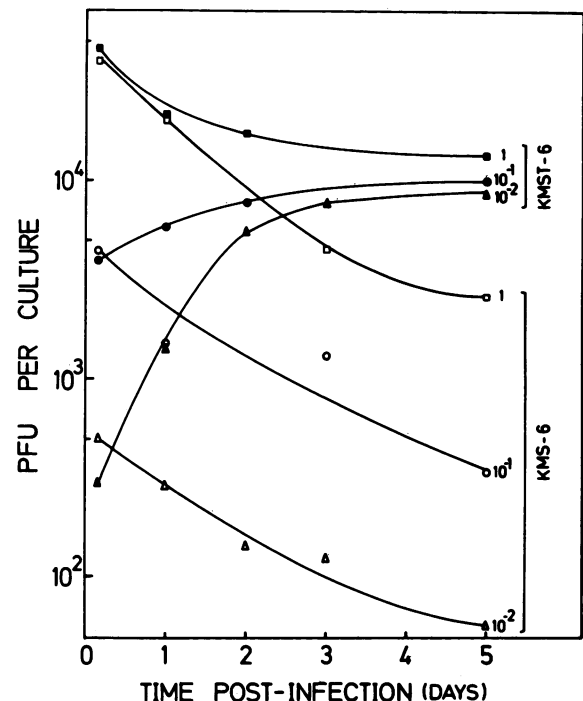

FIG. 5. Fate of infectious MVMp in normal and radiation-transformed human cells. Normal (KMS-6) and gamma-ray-transformed (KMST-6) fibroblasts were infected with MVMp at indicated multiplicities and further incubated. Virus titers were measured as a function of time p.i. Numbers by symbols represent MOI.

or progeny SS viral DNA be detected in Southern blots prepared from these cells. The biological outcome of the abortion of H-1 virus and MVMp replication in most normal cells is to limit viral cytopathogenicity to a mild effect which can be revealed by cloning efficiency tests but from which cultures recover rapidly as virus dilutes out.

Enhanced susceptibility of transformed human fibroblasts to H-1 virus and MVMp. Transformed cells produce higher amounts of MVMp and H-1 virus than their untransformed parent cells do. Viral DNA replication limitations present in normal cells and overcome in transformed derivatives are not known. Since virus uptake occurs with similar efficiencies in both types of cultures, the primary step of the viral life cycle which is stimulated in transformed cells appears to be intracellular. Transformation may conceivably correlate with the activation of a cellular permissiveness function(s) regulating or effecting parvovirus replication. Viral DNA amplification taking place in the transformants seems to proceed as in standard permissive hosts because RF1 and RF2 replicative intermediates accumulate to high levels and form covalent complexes with terminal proteins (2). However, a late step of the virus life cycle, i.e., the synthesis of SS progeny DNA and, consequently, the production of infectious virus, is inefficient or even undetectable in most of the transformants studied. This deficiency occurs despite the capacity of these cells for the assembly of empty capsids (Cornelis et al., unpublished observations). It has been suggested that assembled capsids are subject to modifications which render them able to drive the synthesis of progeny DNA and concomitant encapsidation (21). It is conceivable that such a maturation is defective in many transformants. Alternatively, cellular or viral factors involved in the processing of replicative forms, the generation of progeny viral DNA, or both may be present in limiting amounts in the majority of the transformants analyzed. Hence, transformed human fibroblasts often represent incomplete permissive systems insofar as they support the synthesis of viral DNA (this paper) as well as nonstructural and structural proteins (4, 25; Cornelis et al., unpublished data) while producing little infectious virus. Such nonproductive infections, accompanied by the accumulation of large amounts of viral DNA, are reminiscent of the restrictive interactions observed between TM3 mouse testicular cells and MVMp (10) or between A9 mouse fibroblasts and the lymphotropic strain of minute virus of mice (31, 32, 39). In the latter system, a block to the initiation of DNA transcription may be involved in the restriction (6).

Transformed human fibroblasts are much more sensitive to the lytic action of MVMp and H-1 virus than their normal parent cells are. The enhanced—although not necessarily full—permissiveness of human cell transformants to virus replication is likely to contribute to their killing, although the cytopathic step(s) of the parvoviral life cycle remains to be determined. A potential candidate consists of viral nonstructural proteins because these are suspected of being cytotoxic (28) and are expressed at a higher level by transformed cultures than by their normal progenitors (Cornelis et al., unpublished data).

Correlation between cell transformation and sensitivity to parvoviruses. The contribution of transformation to the sensitization of human fibroblasts to H-1 virus and MVMp remains to be elucidated. It was recently shown that the expression of different oncogenes of cellular (activated human Ha-*ras* 1) or viral (polyomavirus middle T and SV40 large T) origin in established murine fibroblast lines correlates with an increase in their susceptibility to MVMp (19). Interestingly, a production of H-1 virus could also be induced in human embryonic lung cells by coinfecting them with adenovirus 12 (13). It is not known, however, whether the expression of adenovirus early-transforming genes is involved in the latter helper effect. Qualitatively or quantitatively activated oncogenes have been identified in two of the transformants studied here, namely c-Ki-*ras* 2 and c-*myc* in WI38CT-1 cells (18) and N-*ras* in the HT1080 line (15). It is a matter of speculation whether changes at the level of these oncogenes are connected with the enhanced susceptibility of corresponding cells to the parvoviruses.

Since transformation is a multistep process, another interesting question is whether the increase in cell permissiveness correlates with the expression of specific transformation traits. Immortalization does not seem to be a prerequisite for sensitization of human fibroblasts to rodent parvoviruses because the susceptibility of SV40 transformants is enhanced irrespective of whether they have a finite or infinite life span (4). Moreover, established lines of rodent cells can resist parvoviruses and be sensitized upon transformation (19, 20). No correlation was found between the growth rates of human and rodent fibroblasts and their sensitivity to H-1 virus or MVMp (this paper; 19). All transformants studied have aneuploid karyotypes, a characteristic that they share with many tumor cells and that is believed to be of importance for malignant progression (8). In addition, the transformants display alterations in both their morphology and their growth properties (12, 22–24). However, transformed human fibroblasts obtained by SV40 infection or physicochemical treatments clone poorly in soft agar and are not tumorigenic when inoculated in nude mice (22, 23). Similarly, the degree of susceptibility of established murine cell lines to MVMp does not parallel their anchorage-independent growth efficiency (19). Therefore, the potentiation of parvovirus replication and cytopathogenicity does not appear to correlate with cell transformation as a whole but rather with specific and possibly early event(s) of this multistage process, the nature of which remains to be determined. In this respect, autonomously replicating parvoviruses of rodents may prove useful for identification of human cells on the way to malignant transformation and to select for their normal homologs.

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