JOURNAL OF VIROLOGY, Sept. 1992, p. 5651–5657
0022-538X/92/095651-07$02.00/0

Vol. 66, No. 9

Adeno-Associated Virus Sensitizes HeLa Cell Tumors to Gamma Rays

CHRISTIAN WALZ,[1] JÖRG R. SCHLEHOFER,[1]* MICHAEL FLENTJE,[2] VOLKER RUDAT,[3] AND HARALD ZUR HAUSEN[1]

Forschungsschwerpunkt Angewandte Tumorvirologie[1] and Forschungsschwerpunkt Radiologische Diagnostik,[3] Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, and Abteilung für Klinische Radiologie, Radiologische Klinik der Universität Heidelberg,[2] D-6900 Heidelberg, Germany

Received 16 April 1992/Accepted 16 June 1992

Infection with the helper virus-dependent human parvovirus adeno-associated virus (AAV) is known to interfere with cellular transformation in vitro and oncogenesis in vivo. Here we report on sensitization to gamma irradiation by AAV infection of cells in culture and of tumors established from HeLa cells grafted into immunodeficient (nude) mice: infection of HeLa cells with AAV type 2 enhanced cell killing and reduced plating efficiency after irradiation compared with uninfected cells. Similarly, HeLa cell tumors in nude mice displayed a reduced growth rate and were more sensitive to gamma irradiation when the animals were infected with AAV type 2 prior to or after tumor cell inoculation. Since no pathogenicity is known for AAV, the ability of this virus to render radiotherapy of human tumor cells more efficient may up open novel approaches in cancer treatment.

Adeno-associated viruses (AAV) are parvoviruses with a single-stranded DNA genome of about 5,000 bp and depend for their replication on factors provided by helper viruses (adenovirus, herpesvirus, or vaccinia virus) (7, 27, 31) or specific cellular conditions related to factors induced by genotoxic stress (3, 4, 12, 29, 35, 36).

AAV exhibit tumor-suppressive properties in vivo and inhibitory effects on cellular transformation in vitro (for reviews, see references 24 and 25). In rodents, AAV infection interferes with growth of tumors induced by viruses or by cells transformed by viruses or oncogenes (5, 8, 9, 15, 17, 21, 22). Infection with AAV can inhibit cellular transformation by tumor viruses or activated oncogenes (14, 16). In humans infected with AAV, cancer is less frequently observed than in seronegative controls (10, 20, 32).

The mechanism of the tumor-suppressive effects of AAV infections is not understood, but recent reports have implicated interference with transformation-associated cellular functions: in the course of AAV infections, mutagenesis (28) and carcinogen- and virus-induced DNA amplification is inhibited (12, 27, 29, 30) and carcinogen-treated (initiated) cells are selectively killed (13). Furthermore, these viruses perturb the cell cycle (34) and induce differentiation of immortalized cells (18). In addition, sensitization of tumor cells to genotoxic stress by infection with AAV has been observed (1).

Cells of the human cervical carcinoma line HeLa carrying viral DNA integrated in chromosome 17 following in vitro inoculation with AAV type 2 (AAV-2) were shown to exhibit increased sensitivity toward cytotoxic and genotoxic agents such as heat and UV and gamma irradiation in vitro (33).

To assess whether infection with AAV in general could be used to increase the sensitivity of tumors toward gamma irradiation (i.e., to determine whether or not integration is required), we performed experiments with HeLa cells either in culture or after they had produced tumors in nude mice.

Exponentially growing HeLa cells (5×10^5) were infected with AAV-2 (adsorption of 10^3 infectious particles per cell for 30 min). After the times indicated, infected cells were irradiated with ^{60}Co at a source-surface distance of 60 cm and ambient temperature (dose rate, 260 cGy/min) in complete medium. After exposure, cells were harvested by trypsinization, counted, diluted, and plated in triplicate in tissue culture flasks on the basis of an expected survival of about 50 to 100 colonies per flask. After 10 days, cells were stained with crystal violet and screened for colonies of more than 50 cells. Cell survival was calculated by dividing the plating efficiency of the irradiated cells by that of the controls. Survival curves were fitted by using exponential least-square regression. Means and standard deviations of the means of sets of five independent experiments are given. AAV-2 had been propagated in HeLa cells with adenovirus type 2 as a helper virus and purified by centrifugation through a cesium chloride gradient (buoyant density, 1.45 g/cm^3) from disrupted cells after completion of the adenovirus-induced cytopathic effect. The titer of infectious particles was determined by infection of HeLa cells growing in 96-well microplates with serial dilutions of purified virus and subsequent infection with the adenovirus type 2 helper virus (1 PFU per cell). Two days after infection, cells were transferred to nylon membranes by using a dot blot apparatus (Schleicher & Schüll, Dassel, Germany). After lysis of the cells and denaturation of DNA in lysis buffer (1.5 M NaCl, 0.5 M NaOH), filters were hybridized with cloned AAV-2 DNA (pAV-2) radiolabeled by nick translation (23). Autoradiographs of the filters revealed the titer of infectious AAV-2.

In these in vitro (cell culture) assays, HeLa cells infected with AAV-2 revealed decreased survival and reduced plating efficiency after irradiation with gamma rays in comparison with uninfected cells (Fig. 1a). The degree of radiosensitization by AAV-2 increased with the multiplicity of infection (data not shown) and with the time elapsed between virus inoculation and irradiation (Fig. 1b). The observed sensitization of HeLa cells to irradiation is in line with our previous findings that HeLa cells persistently infected with AAV-2 and containing AAV DNA integrated into chromosome 17 were more sensitive to genotoxic stress (e.g., irradiation) than were the parental HeLa cells (33).

* Corresponding author.

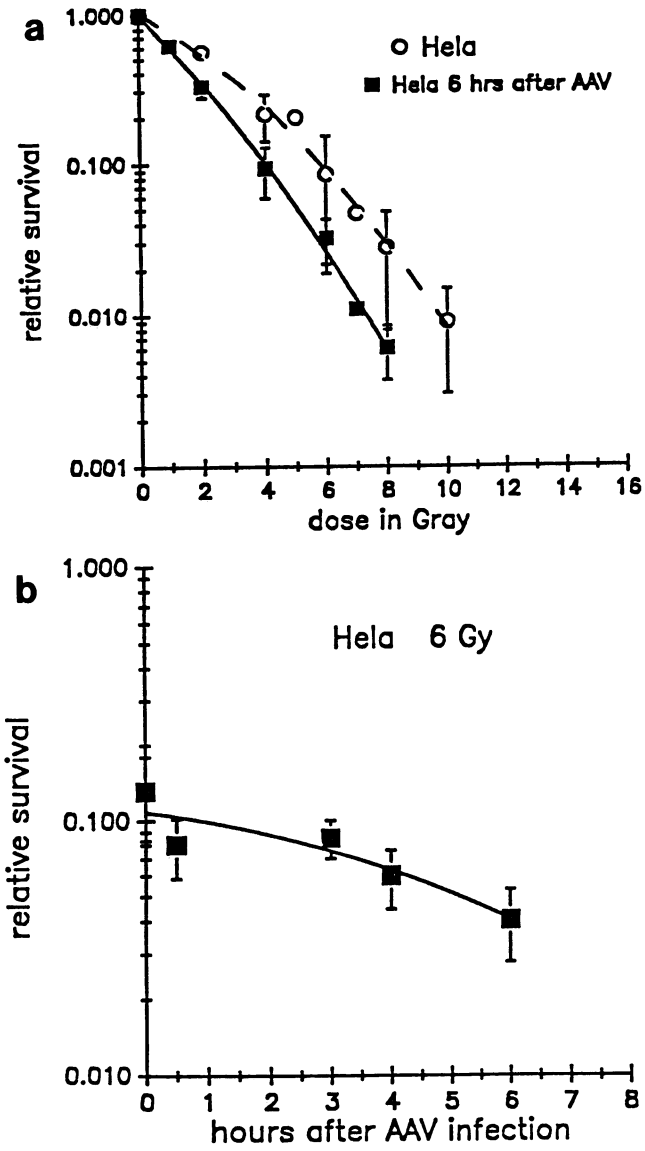

FIG. 1. Sensitization of HeLa cells to gamma rays in vitro by infection with AAV-2. (a) Dose-related sensitivity to irradiation of HeLa cells 6 h after infection with AAV-2. Plating efficiencies (40 to 60%) in nonirradiated controls were not compromised by AAV-2 infection. (b) Kinetics of radiosensitization. Irradiation at different intervals after AAV-2 infection produced increasing radiation-induced cell killing for the first 6 h.

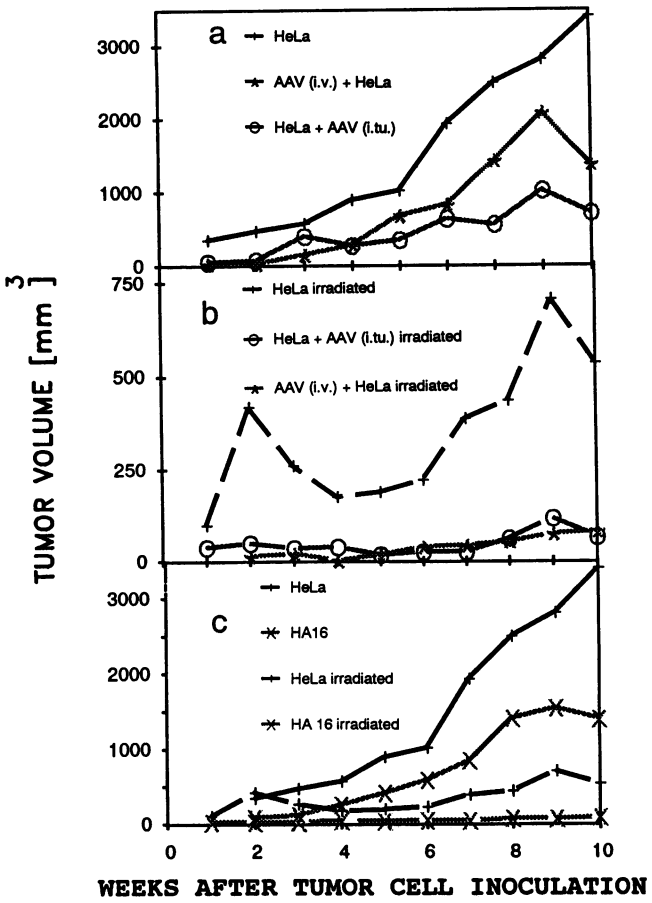

FIG. 2. Sensitization to gamma rays of HeLa cell-derived tumors in nude mice by infection with AAV type 2. (a) Reduced growth rate of tumors from HeLa cells after infection of animals with AAV-2 DNA intravenously 1 week before tumor cell inoculation [AAV (i.v.) + HeLa] or intratumorally 1 week after tumor cell inoculation [HeLa + AAV (i.v.)]. (b) Growth rate of irradiated HeLa cell tumors in uninfected animals (HeLa irradiated) and in animals infected with AAV-2 either intratumorally [HeLa + AAV (i.tu.) irradiated] or intravenously [AAV (i.v.) + HeLa irradiated]. (c) Enhanced sensitivity to irradiation of tumors established in nude mice from HeLa cell clone HA 16 containing integrated AAV-2 DNA (similar results were obtained with two other independently established HeLa cell clones with integrated AAV-2 DNA). HA 16, growth of tumors from clone HA 16; HA 16 irradiated, growth of HA 16-derived tumors in irradiated animals. HeLa tumors in irradiated (HeLa irradiated) and untreated (HeLa) animals are shown for comparison.

To evaluate this AAV-induced radiosensitization in vivo, immunodeficient (nude) mice were infected with AAV-2 prior to or after grafting of HeLa cells subcutaneously into the animals. Exponentially growing HeLa or HA 16 cells (5×10^5 in 100 μl of phosphate-buffered saline) were injected subcutaneously into the right flanks of 7-week-old female nude mice (CD1-*nu/nu*). Animals were purchased from Charles River WIGA (Sulzfeld, Germany), were housed five per cage in isolators, and received food and water ad libitum. We injected 10^{10} tissue culture infective doses of CsCl-purified AAV-2 particles in 100 μl of phosphate-buffered saline into each animal either intravenously [tail vein; AAV (i.v.) + HeLa in Fig. 2] 1 week prior to or intratumorally 1 week after tumor cell inoculation [HeLa + AAV (i.tu.) in Fig. 2]. Before use, virus was heated to 60°C for 30 min to inactivate traces of possibly contaminating adenovirus (used for AAV propagation [cf. Fig. 1]).

Irradiation was performed by exposure of the tumor regions of the animals to gamma rays (10 Gy per session) with a Gammatron II (Siemens, Munich, Germany) at weekly intervals for the 4 weeks following the first week after tumor cell inoculation. Prior to irradiation, the animals were anesthetized (Ketavet, 0.3 mg [Parke-Davis, Berlin, Germany]; Rompun, 1.5 mg per animal [Bayer, Leverkusen, Germany]). In each individual experiment (control, infection, irradiation), five animals were included. Tumor volume was determined weekly by measuring three diameters of the tumors. Ten weeks after tumor cell inoculation, mice were

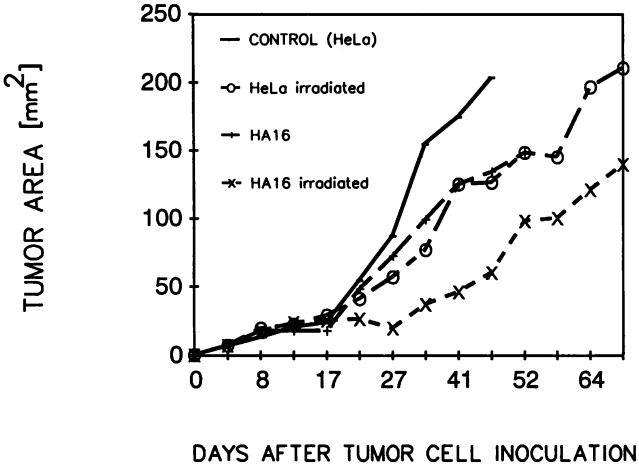

FIG. 3. Growth of HeLa cell tumors and of tumors of HeLa cell-derived clone HA 16 containing integrated AAV-2 DNA after a single irradiation. Cells (5×10^5) were inoculated into the right flanks of immunodeficient nude mice at day 0 (four mice per group). All tumors were irradiated with a single dose of gamma rays (10 Gy) at day 14. Tumor size was determined by measuring two diameters and calculating the tumor area.

sacrificed and tumor tissue and liver, spleen, kidney, brain, lung, heart, bone (including marrow), gut, and ovary tissue samples and blood samples were taken. One half of each sample was frozen immediately in liquid nitrogen for DNA and RNA analysis, and the other half was fixed with formaldehyde (37%) for histological examination. Sections of tissue samples embedded in paraffin were stained with hematoxylin and eosin and analyzed microscopically (see Fig. 5).

Infection with AAV-2 either intravenously prior to or intratumorally 1 week after tumor cell inoculation reduced the growth of tumors to about a sixth of the volume of HeLa cell tumors in uninfected mice (Fig. 2a). Furthermore, tumors in AAV-2-infected mice proved to be more sensitive to gamma irradiation (10 Gy once weekly for 4 weeks) than were control tumors from HeLa cells in uninfected animals (Fig. 2b). After termination of irradiation (after week 5), tumors in AAV-2-infected animals did not resume the initial growth rate, in contrast to HeLa controls. Similarly, intravenous infection of mice 1 week after tumor cell inoculation also produced enhanced radiosensitivity of the tumor tissue (data not shown).

Paralleling the findings on AAV infection, tumors from HeLa cell clones containing AAV-2 DNA integrated into the host cell genome and shown recently to grow more slowly (33) were found to be more sensitive to irradiation than were control (HeLa) tumors (Fig. 2c, which shows the growth of tumors from cell clone HA 16 as one representative clone with integrated AAV DNA) (33).

With the protocol of the in vivo experiments presented here, it is not possible to exclude the possibility that the arrest of tumor growth represents the combined effects of infection plus irradiation. However, experiments with a single irradiation administered to tumors of cells with integrated AAV-2 DNA show a delay in the resumption of tumor growth compared with parental control HeLa cell tumors (clone HA 16, for example [Fig. 3]; a single irradiation treatment was not sufficient to reduce tumor growth). This shift in the growth kinetics suggests enhanced sensitivity of HA 16 cells to irradiation, resulting in complete stoppage of tumor cell proliferation when multiple irradiations were used. (The lag phase prior to resumption of growth was not so obvious when a series of irradiations was administered, since growth was nearly completely stopped under these conditions [Fig. 2b and c].)

DNA was extracted from tissues with phenol-chloroform-isoamyl alcohol by standard procedures (25, 26) and applied to a nylon membrane (GeneScreen; DuPont-NEN, Dreieich, Germany) by using a slot blot apparatus (Schleicher & Schüll). DNA immobilized on nylon membranes was hybridized with radiolabeled AAV-2 DNA in 50 mM NaP_i (pH 7.6)–7% sodium dodecyl sulfate–1 mM EDTA at 68°C for 16 h with gentle agitation. Blots were autoradiographed for 20 min after three washing steps at 68°C in 1% sodium dodecyl sulfate–50 mM NaP_i (pH 7.2)–1 mM EDTA. Cloned DNA of AAV-2 was excised from vector DNA and radiolabeled with

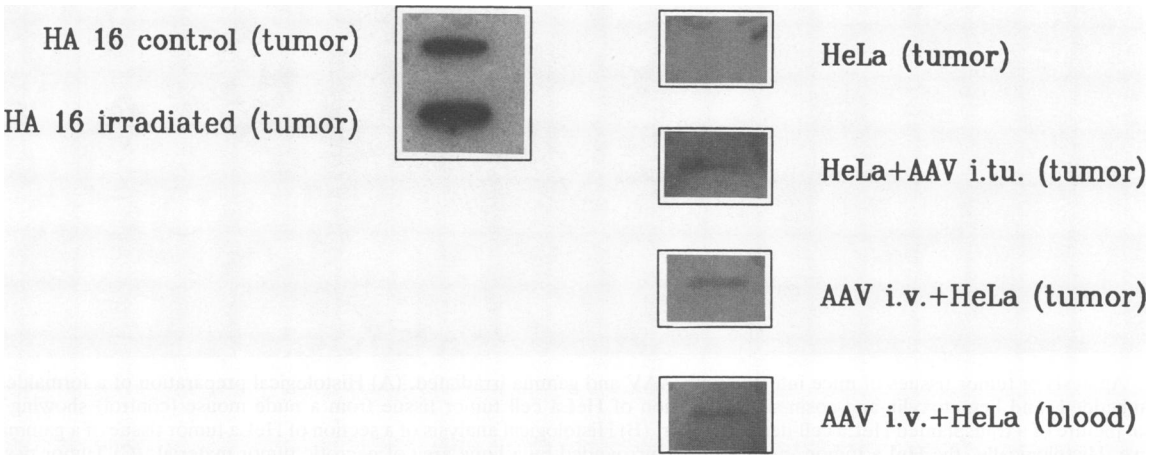

FIG. 4. Slot blot analysis of DNA extracted from HeLa cell tumors and blood from animals (2 days after infection) infected with AAV-2 (intratumorally [i.tu.] or intravenously [i.v.]) and from animals grafted with cells of HeLa-derived clone HA 16 containing integrated AAV-2 DNA. Shown are autoradiograms of 500 ng of cellular DNA applied to nitrocellulose filters, immobilized, and hybridized with [^{32}P]dTTP-labeled AAV-2 DNA. The presence of AAV-2 DNA in tumors and blood from animals examined 2 days after infection is evident. DNA of tumors from animals grafted with an AAV-2 DNA containing HeLa cell clone HA 16 (HA 16 control and HA 16 irradiated) reveals a strong signal after hybridization with AAV-2 DNA.

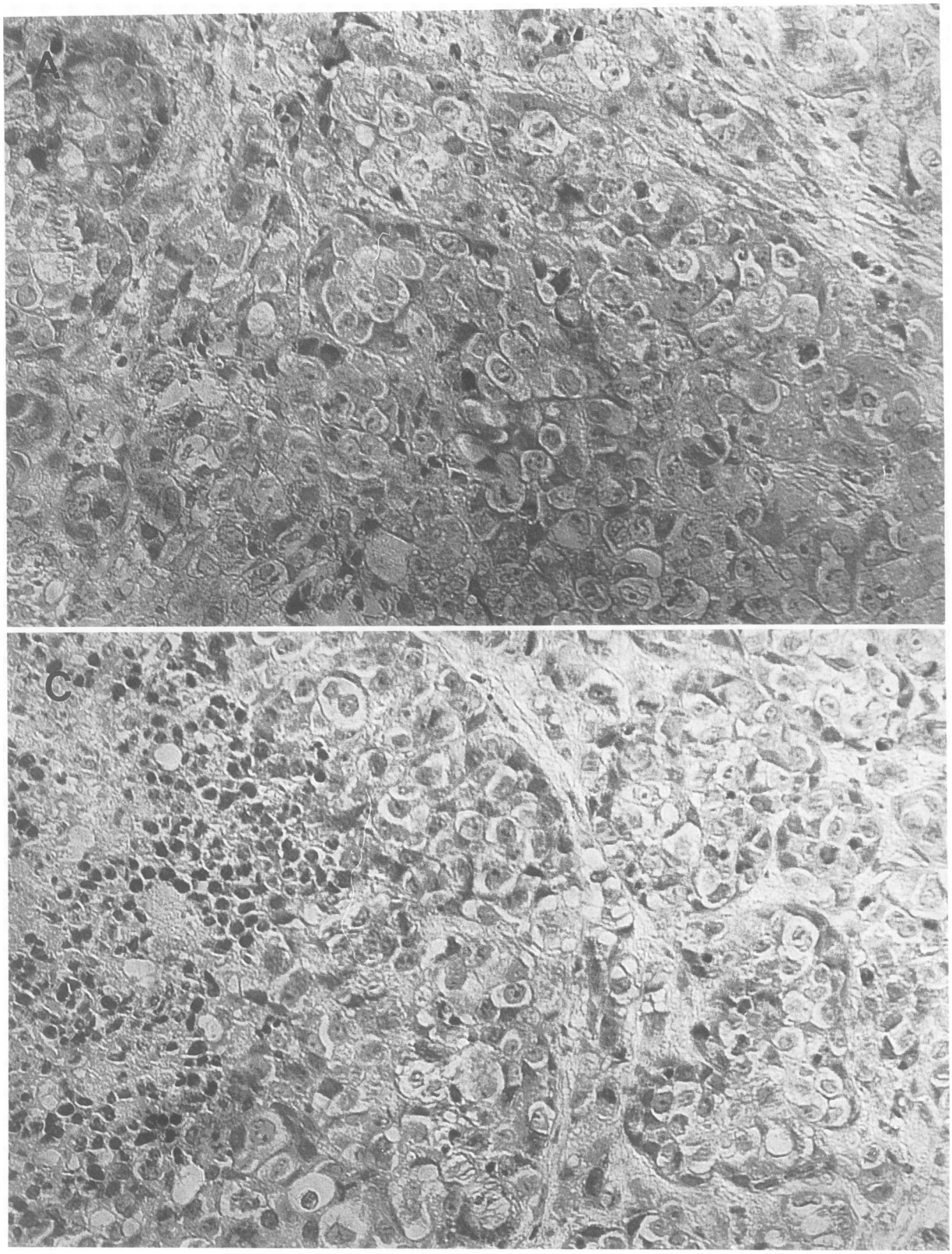

FIG. 5. Analysis of tumor tissues of mice infected with AAV and gamma irradiated. (A) Histological preparation of a formaldehyde-fixed, paraffin-embedded, and hematoxylin-and-eosin-stained section of HeLa cell tumor tissue from a nude mouse (control) showing the typical histological picture of a transplanted HeLa cell-derived tumor. (B) Histological analysis of a section of HeLa tumor tissue of a gamma-irradiated nude mouse. Histologically, the HeLa tumor appears to be surrounded by a large area of necrotic tumor material. (C) Tumor tissue from an AAV-infected mouse: histology of a section of HeLa tumor tissue of a gamma-irradiated nude mouse 1 week after intravenous infection with AAV-2. The histology shows tumor areas with damage due to gamma irradiation adjacent to an area with pyknotic nuclei around an area of necrosis. (D) Histological analysis of a section of HeLa cell tumor tissue of a nude mouse infected with AAV-2 intratumorally 1 week after tumor cell inoculation and then irradiated. The micrograph shows a small tumor area surrounded by pyknotic nuclei within a large necrotic area. Only a few mitotic figures are visible. Irradiation (10 Gy) was performed at the tumor site once a week for 4 weeks beginning 1 week after HeLa cell inoculation (Fig. 2). For analysis of tissues, animals were sacrificed 10 weeks after tumor cell injection.

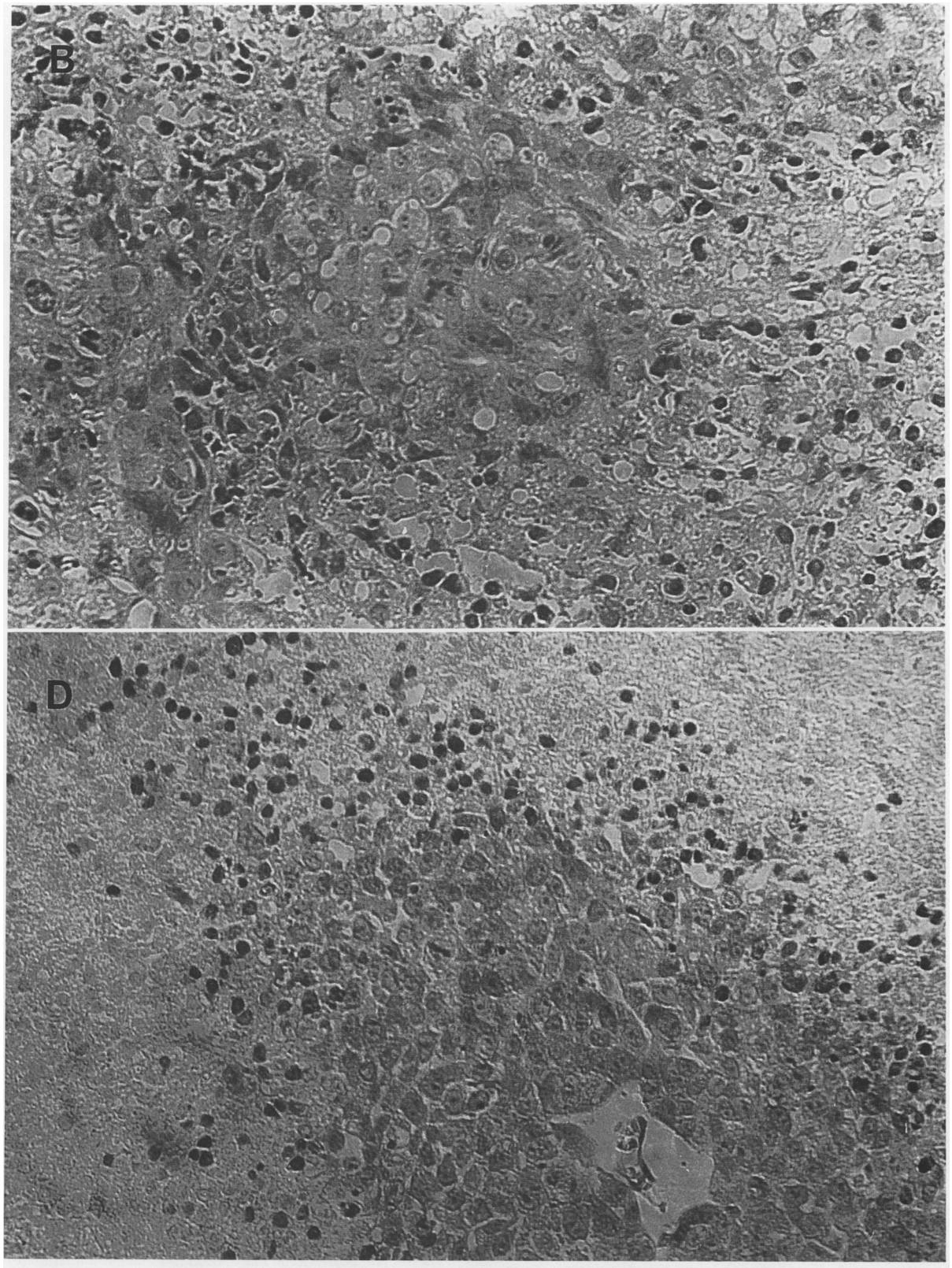

FIG. 5—*Continued.*

[^{32}P]dTTP (Amersham, Braunschweig, Germany) by nick translation (23).

DNA of infecting AAV-2 was demonstrated at up to 2 days after infection in the tumor tissue and in the blood (viremia) of the animals (Fig. 4). In animals grafted with the HeLa cell clone containing integrated AAV-2 DNA (HA 16), AAV DNA was shown to persist (Fig. 4). AAV-2 specific RNA was not detected by Northern (RNA) blot analysis in either tumor tissue or organs (spleen, liver, kidney, heart, lung, ovary, brain, gut, and bone, including marrow) of infected animals (data not shown). Analysis of DNA or RNA specific for human papillomavirus type 18 (present in integrated form in HeLa cells [6]) revealed no modification of the structure of human papillomavirus type 18 DNA or the abundance of human papillomavirus type 18 RNA in the HeLa tumors of animals or in cell cultures infected with AAV and irradiated as described above (data not shown).

Histologically, regression of tumors in AAV-2-infected and irradiated animals was found to be accompanied by lower frequencies of normal and atypical mitoses in the remaining tumor tissue (Fig. 5). Furthermore, these tumors showed a greater proportion of necrosis. No morphological alterations in other organs were detected by histology in AAV-infected and irradiated animals (data not shown).

The mechanism by which AAV sensitizes tumor cells is not clear. With the exception of tumors from clone HA 16 (containing integrated AAV DNA which is transcribed [33]), AAV-specific mRNAs were detected by Northern blot analysis in neither tumor tissues and other organs of infected animals nor infected cell cultures, as expected, without a coinfecting helper virus. Expression of the (early) *rep* genes of AAV has been shown to be essential for regulation of virus replication, interference with cell transformation (14), and inhibition of DNA amplification (11). In the experiments reported here, it is not clear whether the AAV *rep*-encoded proteins were involved in radiosensitization by AAV infection, since the possibility that in regressing tumor cells *rep* genes had been expressed before cell death cannot be excluded. (It has been reported by others [19] that AAV *rep* products can be produced in the absence of helper functions.) It might be possible that the tumor cells analyzed after infection and irradiation were those that did not contain or retain AAV or did not express *rep* and therefore could survive and continue to grow. Another mechanism might reside in the particular structure of the single-stranded DNA molecules of the virus with its terminal hairpin structure due to terminal inverted nucleotide sequence repetitions (3). It might be that structural elements of AAV DNA interfere with cellular factors in tumor cells (e.g., specific binding) and/or with molecules induced by genotoxic stress (e.g., irradiation). This assumption is supported by the fact that tumor suppression in hamster cells was achieved with AAV carrying large deletions (up to 70%) in the genome (9).

Our findings on radiosensitization by AAV of tumors derived from human cells suggest a possible therapeutic use of these nonpathogenic viruses. However, before considering any exploitation of the radiosensitizing effects of these viruses in the treatment of cancer, further investigations are needed to clarify the role of specific structures or functions of AAV in infected tumor cells. In view of the nonpathogenicity of the virus (2–4) and its relation to tumor suppression, however, understanding of the properties of this parvovirus may give rise to novel concepts in tumor therapy.

The technical assistance of M. Ehrbar is gratefully acknowledged. We thank C. Laughlin for plasmid pAV2. We are grateful to V. Amelung for histological analysis of mouse tissues and to H. K. Adldinger for critical reading of the manuscript.

This work was supported by the Deutsche Forschungsgemeinschaft (Schl. 203/3-2).

REFERENCES

1. **Bantel-Schaal, U.** 1991. Infection with adeno-associated parvovirus (AAV) leads to increased sensitivity of mammalian cells to stress. Virology **182:**260–268.
2. **Berns, K. I. (ed.).** 1984. The parvoviruses. Plenum Publishing Corp., New York.
3. **Berns, K. I., and R. A. Bohensky.** 1987. Adeno-associated virus: an update. Adv. Virus Res. **32:**243–306.
4. **Berns, K. I., and W. W. Hauswirth.** 1979. Adeno-associated viruses. Adv. Virus Res. **25:**407–449.
5. **Blacklow, N. R., G. Cukor, S. Kibrick, and G. Quinnan.** 1978. Interaction of adeno-associated viruses with cells transformed by herpes simplex virus, p. 87–98. *In* D. C. Ward and P. Tattersall (ed.), Replication of mammalian parvoviruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Boshart, M., L. Gissmann, H. Ikenberg, A. Kleinheinz, W. Scheurlen, and H. zur Hausen.** 1984. A new type of papillomavirus DNA: its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J. **3:**1151–1157.
7. **Cukor, G., N. R. Blacklow, D. Hoggan, and K. I. Berns.** 1984. Biology of adeno-associated virus, p. 33–66. *In* K. I. Berns (ed.), The parvoviruses. Plenum Publishing Corp., New York.
8. **Cukor, G., N. R. Blacklow, S. Kibrick, and I. C. Swan.** 1975. Effect of adeno-associated virus on cancer expression by herpesvirus-transformed hamster cells. J. Natl. Cancer Inst. **55:** 957–959.
9. **de la Maza, L. M., and B. J. Carter.** 1981. Inhibition of adenovirus oncogenicity in hamsters by adeno-associated virus DNA. JNCI **67:**1323–1326.
10. **Georg-Fries, B., S. Biederlack, J. Wolf, and H. zur Hausen.** 1984. Analysis of proteins, helper dependence and seroepidemiology of a new human parvovirus. Virology **134:**64–71.
11. **Heilbronn, R., A. Bürkle, S. Stephan, and H. zur Hausen.** 1990. The adeno-associated virus *rep* gene suppresses herpes simplex virus-induced DNA amplification. J. Virol. **64:**3012–3018.
12. **Heilbronn, R., J. R. Schlehofer, A. Ö. Yalkinoglu, and H. zur Hausen.** 1985. Selective DNA amplification induced by carcinogens (initiators): evidence for a role of proteases and DNA polymerase alpha. Int. J. Cancer **36:**85–91.
13. **Heilbronn, R., J. R. Schlehofer, and H. zur Hausen.** 1984. Selective killing of carcinogen-treated SV40-transformed Chinese hamster cells by a defective parvovirus. Virology **136:**439–441.
14. **Hermonat, P. L.** 1989. The adeno-associated virus rep 78 gene inhibits cellular transformation induced by bovine papillomavirus. Virology **172:**253–261.
15. **Katz, E., and B. J. Carter.** 1986. Effect of adeno-associated virus on transformation of NIH 3T3 cells by ras gene and on tumorigenicity of an NIH 3T3 transformed cell line. Cancer Res. **46:**3023–3026.
16. **Khleif, S. N., T. Myers, B. J. Carter, and J. P. Trempe.** 1991. Inhibition of cellular transformation by the adeno-associated virus rep gene. Virology **181:**738–741.
17. **Kirschstein, R. L., K. O. Smith, and E. A. Peters.** 1968. Inhibition of adenovirus 12 oncogenicity by adeno-associated virus. Proc. Soc. Exp. Biol. Med. **128:**670–673.
18. **Klein-Bauernschmitt, P., H. zur Hausen, and J. R. Schlehofer.** 1992. Induction of differentiation-associated changes in established human cells by infection with adeno-associated virus type 2. J. Virol. **66:**4191–4200.
19. **Labow, M. A., L. H. Graf, and K. I. Berns.** 1987. Adeno-associated virus gene expression inhibits cellular transformation by heterologous genes. Mol. Cell. Biol. **7:**1320–1325.
20. **Mayor, H. D., S. Drake, J. Stahmann, and D. M. Mumford.** 1976. Antibodies to adeno-associated satellite virus and herpes simplex in sera from cancer patients and normal adults. Am. J. Obstet. Gynecol. **126:**100–104.
21. **Mayor, H. D., G. S. Houlditch, and D. M. Mumford.** 1973.

Influence of adeno-associated satellite virus on adenovirus-induced tumors in hamsters. Nature (London) New Biol. **241:** 44–46.

22. **Ostrove, J. M., D. H. Duckworth, and K. I. Berns.** 1981. Inhibition of adenovirus-transformed cell oncogenicity by adeno-associated virus. Virology **113:**521–533.
23. **Rigby, P. W., C. Rhodes, and P. Berg.** 1977. Labelling desoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113:**237–252.
24. **Rommelaere, J., and J. J. Cornelis.** 1991. Antineoplastic activity of parvoviruses. J. Virol. Methods **33:**233–251.
25. **Rommelaere, J., and P. Tattersall.** 1990. Oncosuppression by parvoviruses, p. 41–57. *In* P. Tijssen (ed.), Handbook of parvoviruses, vol. II. CRC Press, Inc., Boca Raton, Fla.
26. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
27. **Schlehofer, J. R., M. Ehrbar, and H. zur Hausen.** 1986. Vaccinia virus, herpes simplex virus and carcinogens induce DNA amplification in a human cell line and support replication of a helper virus dependent parvovirus. Virology **152:**110–117.
28. **Schlehofer, J. R., and R. Heilbronn.** 1990. Infection with adeno-associated virus type 5 inhibits mutagenicity of herpes simplex virus type 1 or 4-nitroquinoline-1-oxide. Mutat. Res. **244:**317–320.
29. **Schlehofer, J. R., R. Heilbronn, B. Georg-Fries, and H. zur Hausen.** 1983. Inhibition of initiator-induced SV40 gene amplification in SV40-transformed Chinese hamster cells by infection with a defective parvovirus. Int. J. Cancer **32:**591–595.
30. **Schmitt, J., J. R. Schlehofer, K. Mergener, L. Gissmann, and H. zur Hausen.** 1989. Amplification of bovine papillomavirus DNA by N-methyl-N′-nitro-N′-nitrosoguanidine, ultraviolet irradiation or infection with herpes simplex virus. Virology **171:**73–81.
31. **Siegl, G., R. C. Bates, K. I. Berns, B. J. Carter, D. C. Kelly, E. Kurstak, and P. Tattersall.** 1985. Characteristics and taxonomy of Parvoviridae. Intervirology **23:**61–73.
32. **Sprecher-Goldberger, S., L. Thiry, N. Lefèbvre, D. Dekegel, and F. de Halleux.** 1971. Complement-fixation antibodies to adenovirus-associated viruses, adenoviruses, cytomegaloviruses and herpes simplex viruses in patients with tumors and in control individuals. Am. J. Epidemiol. **94:**351–358.
33. **Walz, C., and J. R. Schlehofer.** 1992. Modification of some biological properties of HeLa cells containing adeno-associated virus DNA integrated into chromosome 17. J. Virol. **66:**2990–3002.
34. **Winocour, E., M. F. Callaham, and E. Huberman.** 1988. Perturbation of the cell cycle of adeno-associated virus. Virology **167:**393–399.
35. **Yakobson, B., T. Koch, and E. Winocour.** 1987. Replication of adeno-associated virus in synchronized cells without the addition of a helper virus. J. Virol. **61:**972–981.
36. **Yalkinoglu, A. Ö., R. Heilbronn, A. Bürkle, J. R. Schlehofer, and H. zur Hausen.** 1988. DNA amplification of adeno-associated virus as a response to cellular genotoxic stress. Cancer Res. **48:**3123–3129.