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Theiler's Murine Encephalomyelitis Virus Kills Restrictive but Not Permissive Cells by Apoptosis

M. L. JELACHICH AND H. L. LIPTON*

Division of Neurology, Evanston Hospital, and Departments of Neurology and Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois 60201

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Theiler's murine encephalomyelitis viruses (TMEV), genus *Cardiovirus*, family *Picornaviridae*, are natural enteric pathogens of mice which cause central nervous system demyelination similar to that seen in multiple sclerosis. TMEV can be classified into two groups based on neurovirulence: a highly virulent group, e.g., GDVII virus, and a less virulent group, e.g., BeAn virus. Both viruses, depending on the multiplicity of infection, produced cytopathology in BSC-1 cells similar to that in BHK-21 cells. Since apoptosis has been reported as a mechanism of cell death after infection with many viruses, we examined infected BHK-21 and BSC-1 cells for morphological and biochemical changes consistent with apoptosis. Only the restrictive BSC-1 cells showed evidence of nuclear morphology and internucleosomal DNA degradation indicative of apoptosis. Interestingly, the more virulent GDVII virus was at least 50-fold more efficient in inducing apoptosis than the less virulent BeAn virus. This difference was not due to greater GDVII viral RNA replication or production of infectious virus, since the two viruses were similarly restricted in BSC-1 cells. Apoptosis in BSC-1 cells appears to be triggered by a cytoplasmic event, since inactivation of GDVII viral RNA by UV light abolished the ability of the virus to induce apoptosis. The possible role of apoptosis in the pathogenesis of TMEV infection in mice, especially virus persistence in central nervous system macrophages, is discussed.

Theiler's murine encephalomyelitis viruses (TMEV), members of the genus *Cardiovirus* in the family *Picornaviridae*, are natural enteric pathogens that cause central nervous system (CNS) disease in mice. The TMEV can be divided into two groups based on neurovirulence characteristics after intracerebral inoculation of mice: (i) the highly virulent viruses, represented by GDVII, which produce fatal encephalitis in mice, and (ii) the less virulent strains, such as DA and BeAn, which produce a persistent CNS infection associated with chronic, inflammatory demyelinating disease. TMEV persistence leads to immunopathologic damage of myelin, mediated by major histocompatibility class II-restricted Th1 T cells directed to virus epitopes rather than to host neuroantigens (2, 6, 7, 20, 21, 38).

Viruses from both TMEV neurovirulence groups replicate to high titer in permissive BHK-21 cells, whereas only low levels of virus are produced in restrictive BSC-1 cells (13, 33). In one-step growth experiments, TMEV infection of BHK-21 cell monolayers leads to typical cytopathic effects (CPE; rounding and loss of adherence) 6 to 12 h after infection, with a four- to fivefold $\log_{10}$ increase in virus titer. In contrast, Sturman and Tamm (33) reported in 1966 that GDVII virus infection of BSC-1 cells resulted in limited CPE with no measurable virus production. In 1991, Kilpatrick and Lipton (13) found that BeAn virus did not produce CPE in BSC-1 cells at low multiplicities of infection (MOIs) but did cause focal areas of nonprogressive CPE following transfection of virus RNA. In this report, we show that TMEV does infect BSC-1 cells, albeit in a highly restricted manner.

A number of DNA and RNA viruses, including chicken anemia virus (25), varicella-zoster virus (31), human immunodeficiency virus (14), Sindbis virus (15, 36), influenza virus (9), and reovirus (35), have been reported to trigger apoptotic cell death as the result of productive infection. These viruses use the cell's apoptotic machinery to induce their CPE. In contrast, other viruses, such as adenovirus (26, 27), poxviruses (1, 10), Epstein-Barr virus (8), baculovirus (4), and African swine fever virus (24), have evolved mechanisms to subvert the apoptotic process, presumably to allow cell survival and continued virus replication. For these viruses, apoptosis seems to be an altruistic cellular defense mechanism to limit virus replication.

Here we report that TMEV induces apoptosis in restrictive BSC-1 cells but not in permissive BHK-21 cells. Moreover, the highly virulent GDVII strain is at least 50-fold more efficient than the less virulent BeAn strain in inducing apoptosis in BSC-1 cells. This difference is not due to greater GDVII RNA replication or production of infectious virus, since GDVII and BeAn are similarly restricted in BSC-1 cells. The apoptotic process appears to be triggered from interactions in the cytoplasm and may play a role in the pathogenesis of the disparate CNS diseases produced by TMEV in mice.

MATERIALS AND METHODS

Cells and viruses. BSC-1 cells, derived from African green monkey kidneys, were purchased from the American Type Culture Collection and maintained in minimum essential medium (GIBCO) containing 2 mM L-glutamine, 0.1 mM supplemental nonessential amino acids, and 10% fetal bovine serum (FBS; HyClone). BHK-21 cells, originally derived from baby hamster kidney cells, were purchased from the American Type Culture Collection and maintained in Dulbecco's minimum essential medium (DMEM; GIBCO) containing 2 mM L-glutamine, 7.5% tryptose phosphate, and 7.5% FBS. The origins and passage histories of GDVII and BeAn viruses have been described elsewhere (29). Both viruses were plaque purified twice, and stocks were prepared in BHK-21 cells after two additional passages. Virus titers were determined by standard plaque assay on BHK-21 cells (29).

Virus infections. After virus adsorption at the indicated MOIs for 45 min at 24°C, cell monolayers were washed twice with phosphate-buffered saline (PBS) containing 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$ and incubated with DMEM containing 5% FBS for the times indicated at 37°C.

Electron microscopy. Cells were fixed with Karnofsky's buffer and 1% OsO_4, stained in uranyl acetate for 30 min followed by 2.5 min in lead citrate, and embedded in Epon-812. Ultrathin sections were examined with a JEOL 100CX electron microscope.

* Corresponding author. Mailing address: Division of Neurology, Evanston Hospital, 2650 Ridge Ave., Evanston, IL 60201. Phone: (708) 570-2168. Fax: (708) 570-1568. Electronic mail address: hllipton@merle.acns.nwu.edu.

Flow cytometry. Staining for flow cytometry was done as described previously (11). Briefly, 10^6 cells were blocked with 10% goat serum, incubated with a 1:1,000 dilution of polyclonal rabbit anti-BeAn serum or normal rabbit serum for 30 min, and then incubated with a 1:200 dilution of secondary fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit antibody (Cappel) for 30 min. Cells were fixed in 1% paraformaldehyde and analyzed on a FACScan (Becton Dickinson, Palo Alto, Calif.). Data were evaluated by using the Consort 30 and LYSIS I computer programs.

Labeling of viral proteins and immunoprecipitation. Labeling with L-[^{35}S]methionine and immunoprecipitation of viral proteins were done as described previously (11). Briefly, BHK-21 cells were incubated with 100 mCi of L-[^{35}S]methionine (100 Ci/mmol; ICN) for 4 to 6 h, and BSC-1 cells were incubated for 16 to 24 h at 37°C. Lysates from 10^6 cells were prepared in radioimmunoprecipitation assay buffer and centrifuged at 1,000 rpm at 4°C for 10 min, and supernatants were immunoprecipitated with 5 μl of undiluted polyclonal rabbit anti-BeAn serum described above (this amount of antibody is not limiting in these experiments). Immunoprecipitated samples were solubilized in sample buffer and electrophoresed on sodium dodecyl sulfate–12% polyacrylamide gels.

Viral RNA replication measured by [^{3}H]uridine incorporation. BSC-1 cells were plated at 10^4 cells per well in 96-well plates. After virus adsorption, 100 μl of medium containing 5 μg of actinomycin D per ml and 10 μCi of [^{3}H]uridine (15 to 25 Ci/mmol; ICN) per ml was added. Cells were incubated for the indicated times at 37°C, harvested on a PHD Cell Harvester (Cambridge Technologies, Watertown, Mass.), and counted in a Beckman scintillation counter (LS5000TD; Beckman Instruments, Palo Alto, Calif.). Data were calculated and graphed by using Cricket GraphIII software.

MTT assay. The 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay is based on the ability of viable cells to reduce MTT to form a colored formazan product (11). Briefly, cells maintained for 18 to 24 h after infection were incubated with 50 μl of MTT (1 mg/ml) for the last 3 to 4 h. Formazan crystals produced by viable cells were dissolved in 50 μl of dimethyl sulfoxide, and the optical density at 560 nm was read on a UVMax microplate reader (Molecular Diagnostics, Palo Alto, Calif.). Quadruplicate samples were averaged, and the percentage of control values was calculated. The standard error of the mean was <10% in all cases.

DNA isolation and 3′ end labeling. Cells were lysed in TTE buffer (10 mM Tris-HCl [pH 7.4], 0.2% Triton X-100, 1 mM EDTA), and intact chromatin was separated from fragmented DNA by centrifugation. After ethanol precipitation, soluble and insoluble DNA fractions were electrophoresed on 2% agarose gels for DNA degradation. For 3′ end labeling, DNA samples were phenol chloroform extracted, ethanol precipitated, and resuspended in a 20-μl reaction mixture containing 75 mM $CoCl_2$, 25 U of terminal transferase, and 50 μCi of [^{32}P]dCTP. After incubation for 60 min at 37°C, terminal transferase was heat inactivated for 10 min at 65°C and the DNA was resolved on 2% agarose gels.

UV irradiation of virus. GDVII virions, in 3 ml of PBS containing 0.1% bovine serum albumin in a 100-mm-diameter petri dish on ice, were irradiated at a distance of 30 cm with a General Electric G15T8 germicidal light. Samples (500 μl) were withdrawn at 0, 1, 2, 4, 8, and 16 min of UV light exposure. All virus samples bound equivalently to BSC-1 cells when analyzed by flow cytometry; however, only virus exposed for 0 min showed no reduction in virus titer.

RESULTS

BSC-1 cells develop CPE after adsorption with GDVII virus. BSC-1 cells have been described as either restrictive (33) or receptor negative (13) for TMEV infection. We found that BSC-1 cells to which the less virulent BeAn virus was adsorbed at an MOI of 10 showed no CPE even after prolonged incubation, whereas BSC-1 cells adsorbed with the highly virulent GDVII virus at an MOI of 10 rounded up and detached from the monolayer within 18 to 24 h (Fig. 1A and B). These morphologic features in BSC-1 cells were characteristic of the TMEV-induced CPE seen in permissive BHK-21 cells.

GDVII virus induces apoptosis in BSC-1 cells. To characterize the CPE, BSC-1 cells were stained with 4′,6-diamidino-2-phenylindole (DAPI), a nuclear stain. More than 50% of BSC-1 cells adsorbed with GDVII virus (MOI of 10) contained condensed chromatin and apoptotic bodies (Fig. 1D), features described for apoptosis (28). These nuclear changes were not observed in control (Fig. 1C) or BeAn virus-adsorbed BSC-1 cells (not shown) or in TMEV-infected BHK-21 cells (Fig. 1E and F). The ultrastructure of BSC-1 cells adsorbed with GDVII virus (MOI of 10) confirmed that BSC-1 cells were undergoing apoptotic cell death, as indicated by chromatin that was condensed and sequestered to the periphery of the nucleus (Fig. 2B). These changes were not observed in control (Fig. 2A) or BeAn virus-adsorbed BSC-1 cells (Fig. 2C) or in TMEV-infected BHK-21 cells (Fig. 2E and F).

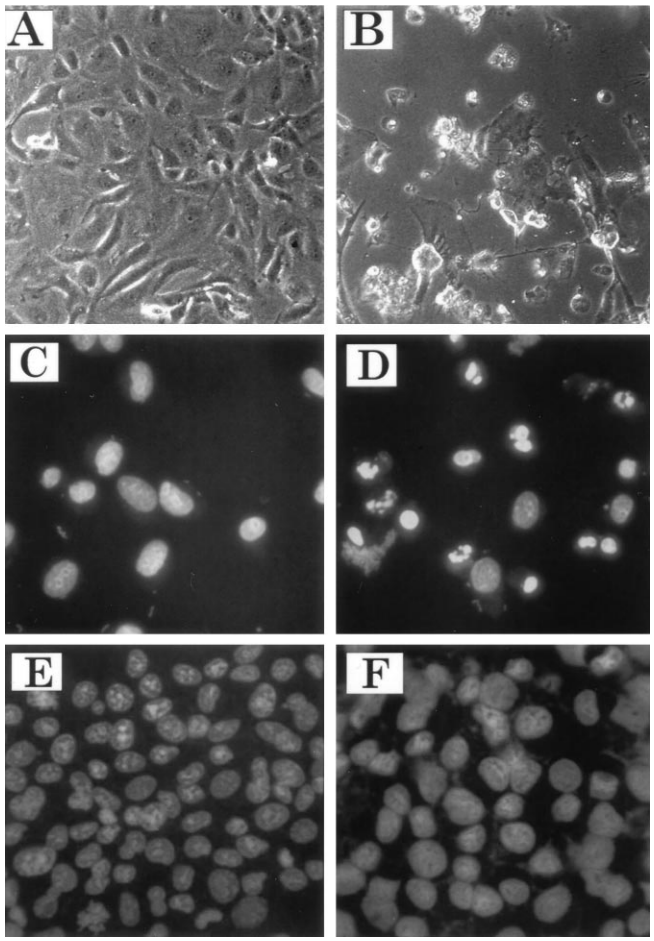

FIG. 1. Morphology of mock-infected (A) and GDVII-infected (B) BSC-1 cells and DAPI staining of mock-infected (C) and GDVII-infected (D) BSC-1 cells and of mock-infected (E) and GDVII-infected (F) BHK-21 cells. GDVII virus was adsorbed to 10^6 BSC-1 cells per well in 35-mm-diameter six-well plates at an MOI of 10 for 45 min at 24°C and then incubated for 15 h at 37°C. Uninfected cells are flattened and adherent to the plastic (A), whereas GDVII virus-infected cells are rounded and detaching from the plastic (B). Methanol-fixed cells were stained with DAPI (0.5 mg/ml) for 15 min at 37°C, washed twice with methanol, and examined with a Zeiss Axioscope with epifluorescence light excitation and Zeiss narrow excitation and emission band-pass filters (excitation G365, dichroic FT395, barrier LP420) for DAPI. Magnification for all panels, ×20.

A biochemical hallmark of apoptosis is internucleosomal DNA degradation which is visualized by ethidium bromide staining of DNA following gel electrophoresis (5). As shown in Fig. 3A, DNA of BSC-1 cells adsorbed with GDVII virus (MOI of 10) showed the typical internucleosomal-size laddering seen with DNA degradation. This pattern was not observed in the DNA extracted from control and BeAn virus-adsorbed BSC-1 cells (MOI of 10) (Fig. 3A) or in TMEV-infected BHK-21 cells examined at 4, 5, 8, and 24 h after infection (Fig. 3B shows only results at 24 h). The absence of degradation of BSC-1 DNA following the adsorption with BeAn virus (MOI of 10) was confirmed by the more sensitive method of 3′ end labeling of DNA with terminal transferase; GDVII-treated BSC-1 cells showed DNA laddering (Fig. 3C). Thus, it initially appeared that only GDVII virus was able to induce apoptosis in BSC-1 cells.

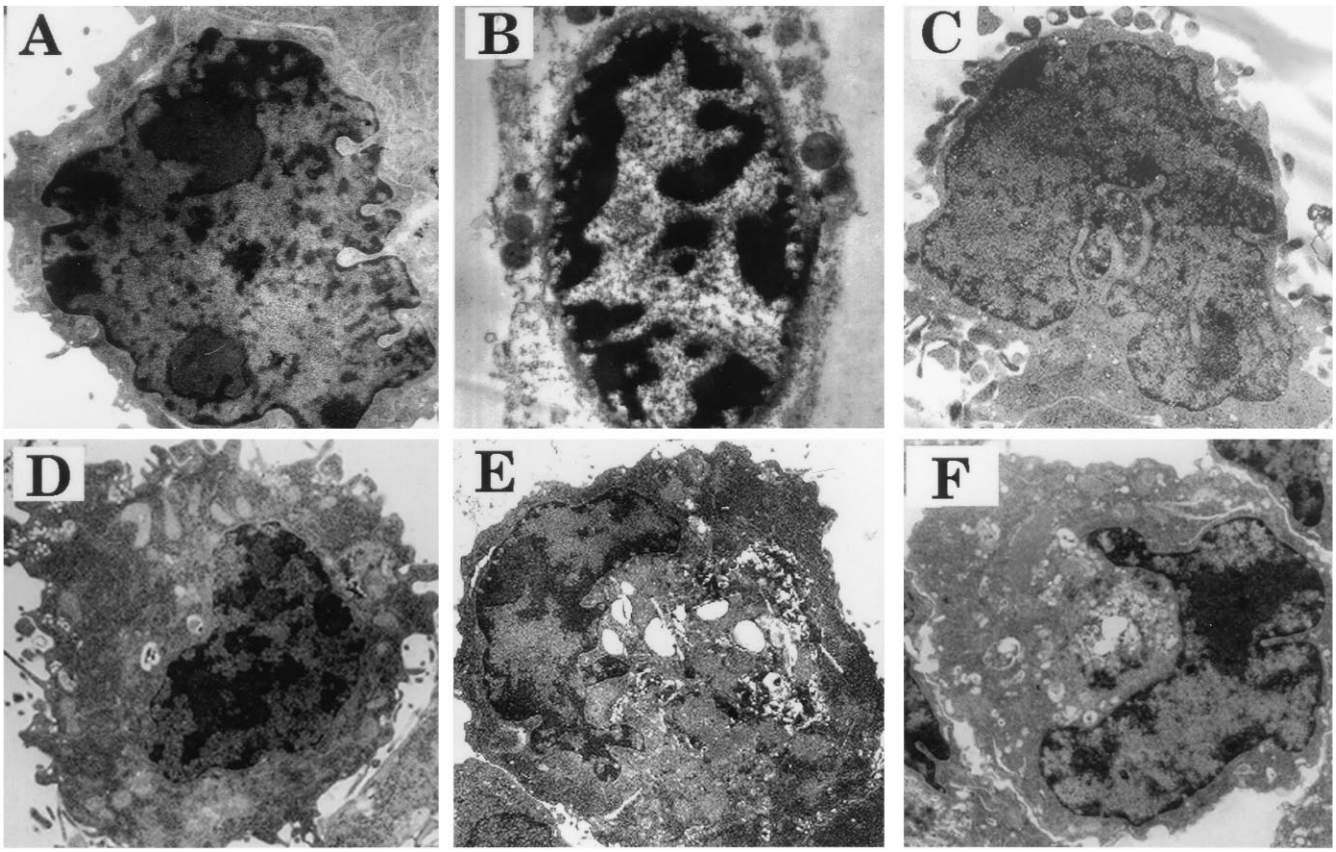

FIG. 2. Electron micrographs of BSC-1 (A to C) and BHK-21 (D to F) cells. Cells were mock infected (A and B), infected with GDVII (B and E) or BeAn (C and F) virus at an MOI of 10 for 45 min at 24°C, and incubated for 12 h (BSC-1 cells) or 6 h (BHK-21 cells) at 37°C. Cells were fixed in Karnofsky's buffer, postfixed in 1% OsO_4, and embedded in Epon. Sections (1 μm) were stained with uranyl acetate and lead citrate and examined with a JEOL 100CX electron microscope. Magnifications: ×13,900 (A), ×25,600 (B), and ×11,500 (C to F).

BeAn virus induces apoptosis but is less efficient than GDVII virus. To compare the abilities of the two TMEV strains to induce apoptotic cell death, the MTT assay, which measures mitochondrial activity, was used to quantitate cell viability. The temporal profile of MTT activity after infection at an MOI of 10 (Fig. 4A) showed that apoptosis by GDVII virus began after 8 h, while that of BeAn virus began after 15 h.

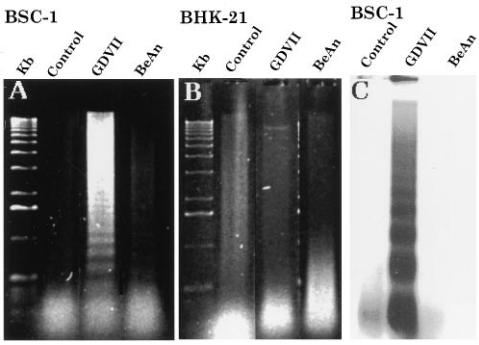

FIG. 3. Ethidium bromide staining of DNA from BSC-1 (A) and BHK-21 cells (B) and 3′ end labeling of DNA from BSC-1 cells (C). Cells were adsorbed with medium alone or with GDVII or BeAn virus at an MOI of 10. After 18 h, cells were lysed and intact nuclei removed by centrifugation. DNA was extracted with phenol-chloroform, precipitated with ethanol, and resolved on a 2% agarose gel. Results are representative of five separate experiments. For 3′ end labeling, cells were incubated with terminal transferase and [^{32}P]dCTP as described in Materials and Methods. Kb, size ladder.

This result for GDVII virus was confirmed by 3′ end labeling DNA with terminal transferase (Fig. 4C), after which DNA degradation clearly appeared at 12 h. Thus, unlike the rapid DNA degradation reported for some stimuli which begins within 4 h, TMEV-induced apoptosis requires more time to develop (37). At MOIs of 10 or more, BeAn virus killed a substantial proportion of BSC-1 cells (Fig. 4B); however, DNA degradation by ethidium bromide staining was observed only at MOIs of >50 PFU per cell (data not shown), suggesting that the laddering of DNA as visualized by ethidium bromide staining is a less sensitive measure of apoptosis than the MTT assay. The dose-response curves shown in Fig. 4B demonstrated that GDVII virus was at least 50-fold more efficient than BeAn in inducing apoptosis, as indicated by an arbitrary endpoint determination of mean 50% cell death. Together, these findings indicate that in restrictive BSC-1 cells, GDVII virus was much more efficient than BeAn virus in inducing apoptotic cell death.

TMEV production in restrictive BSC-1 cells. TMEV RNA replication was measured by incorporation of [^{3}H]uridine in the presence of actinomycin D in BSC-1 and BHK-21 cells. At an MOI of 10, replication of both GDVII and BeAn virus RNA was substantially reduced and the kinetics were delayed compared with results for BHK-21 cells (Fig. 5A). The difference in peak virus RNA levels for the two viruses was less than twofold in BSC-1 cells. Increasing the MOI to 100 in BSC-1 cells did not increase TMEV RNA replication (not shown). Determination of virus yields by standard plaque assay after infection of BSC-1 cells at an MOI of 10 revealed that both

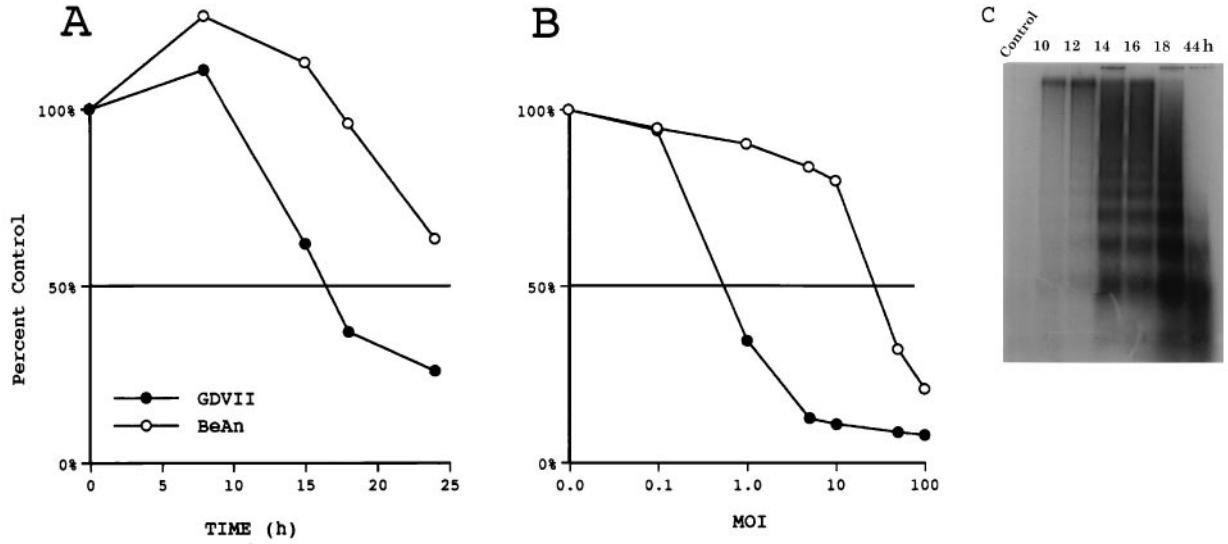

FIG. 4. Temporal kinetics (A) and dose response at 24 h (B) of apoptosis measured by the MTT assay and 3′ end labeling of DNA (C). BSC-1 cells were infected with either GDVII or BeAn virus at an MOI of 10 (A) or at indicated MOIs (B), MTT was added for the last 3 h of incubation at 37°C, the formazan product of mitochondrial cleavage of MTT was resuspended in 50 μl of dimethyl sulfoxide, and the optical density at 560 nm was read. Quadruplicate samples were averaged, and results are expressed as percentages of values for uninfected controls. The standard error of the mean was always less than 10%. For 3′ end labeling, cells were infected with GDVII virus at an MOI of 10 and incubated with terminal transferase and [^{32}P]dCTP as described in Materials and Methods.

TMEV strains produced only 0.5 to 1.0 PFU per BSC-1 cell (mean virus titers at 24 h were 1.1×10^5 and 5×10^4 PFU/10^6 cells for GDVII and BeAn viruses, respectively [3 experiments]). Flow cytometry demonstrated cytoplasmic virus antigen(s) in BSC-1 cells at 15 h postinfection, but the peak fluorescence intensity was substantially less than that seen in BHK-21 cells at 8 h postinfection (Fig. 5B). Since TMEV infection in BSC-1 cells did not inhibit host cell protein synthesis (not shown), [^{35}S]methionine-labeled infected BSC-1 cell lysates were immunoprecipitated with polyclonal rabbit antiserum to TMEV to identify the capsid proteins. As shown in Fig. 5C, TMEV capsid proteins were detected in BSC-1 as well as in BHK-21 cells infected with either virus. Further, the amount of immunoprecipitated capsid proteins from infected BSC-1 cells decreased with increasing MOI (not shown). The decline in the amount of immunoprecipitated capsid proteins from GDVII virus-infected cells began at an MOI of 1, while that from BeAn virus-infected cells began at an MOI of 20 (not

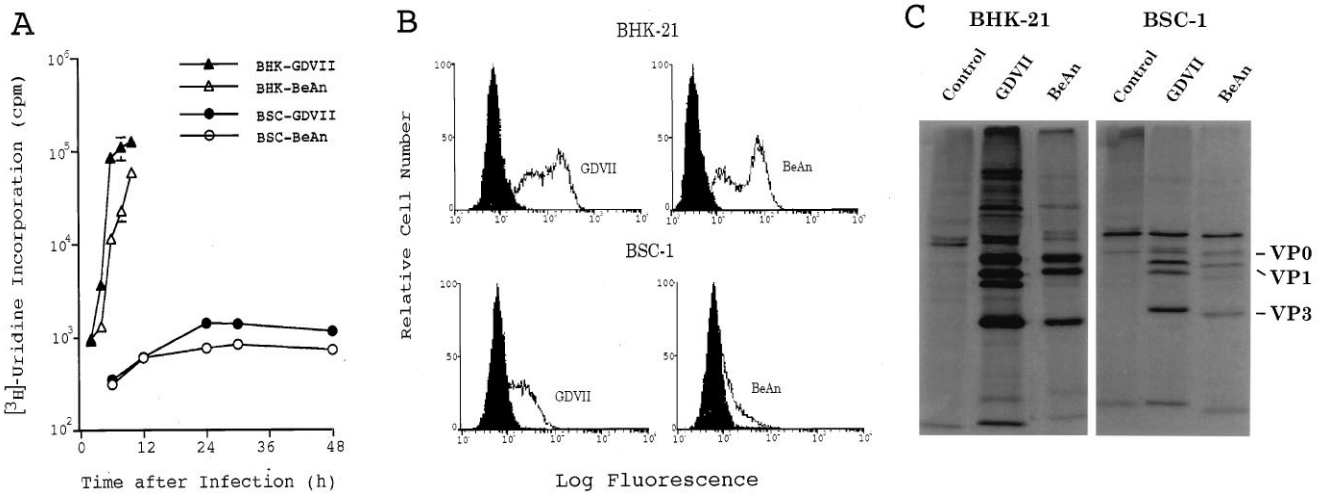

FIG. 5. (A) TMEV RNA replication after infection at an MOI of 10. BSC-1 cells (10^4) were incubated with 5 μg of actinomycin D per ml and 1 μCi of [^{3}H]uridine in 100 μl of DMEM supplemented with 5% FBS for the indicated times. Samples were harvested and assessed for radioactivity in a scintillation counter. Data are given as means and standard deviations of quadruplicate samples. (B) Flow cytometry of TMEV antigens present in the cytoplasm 15 h after adsorption of GDVII or BeAn virus at an MOI of 5. Cells were stained for surface or cytoplasmic antigen with polyclonal rabbit anti-BeAn serum (1:1,000 dilution followed by FITC-conjugated goat anti-rabbit immunoglobulin serum [1:200 dilution]) and analyzed on a BD FACScan using LYSIS I software. When surface staining is taken into account, 22% of GDVII virus-infected cells and 40% of BeAn virus-infected cells were positive for cytoplasmic antigen. (C) Immunoprecipitates of synthesized TMEV capsid proteins. GDVII or BeAn virus was adsorbed to BSC-1 or BHK-21 cells at an MOI of 5. After 4 h of incubation at 37°C, cells were incubated in methionine-deficient medium for 60 min, 100 μCi of [^{35}S]methionine was added, and incubation was continued for 4 h (BHK-21 cells) or 20 h (BSC-1 cells). Cells were lysed in radioimmunoprecipitation assay buffer at 4°C and precleared with normal rabbit serum, and 10^6 cell equivalents were immunoprecipitated with 5 μl of polyclonal rabbit antiserum to BeAn virus. Immunoprecipitated proteins were harvested with protein A-Sepharose and resolved on a 12% polyacrylamide gel. The capsid proteins VP0, VP1, and VP3 are indicated.

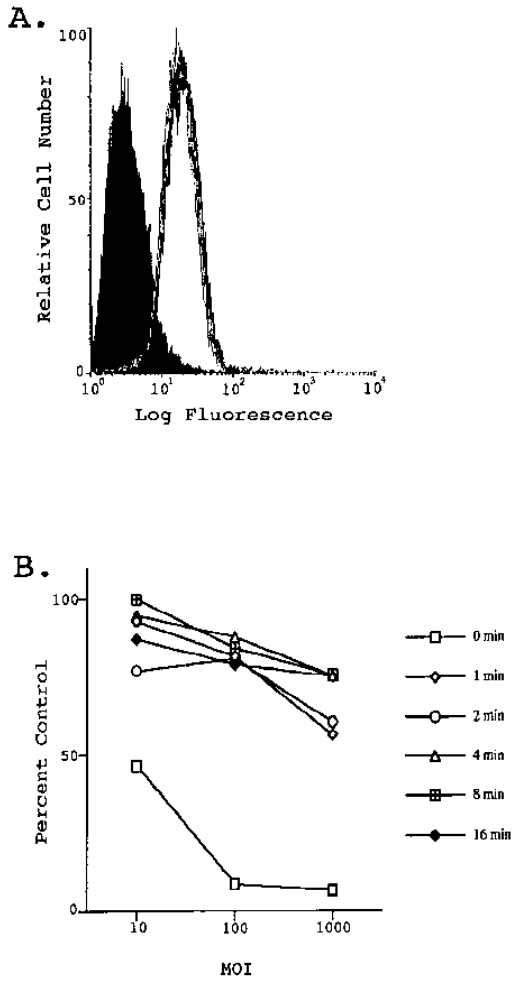

FIG. 6. UV inactivation of GDVII virus. (A) Flow cytometry of BSC-1 cells adsorbed with medium or GDVII virus at an MOI of 100 exposed to UV light for 0, 1, 2, 4, 8, and 16 min. Cells were stained with polyclonal rabbit anti-BeAn serum and goat FITC-conjugated anti-rabbit serum as described for Fig. 5. UV treatment for the indicated times does not affect virus binding to the cell surface. (B) MTT assay of BSC-1 cells infected with GDVII virus exposed to UV light for 0 to 16 min markedly altered the ability of the virus to induce apoptosis. The MTT assay was performed as described for Fig. 4.

shown). In contrast, a threshold of precipitable capsid proteins was reached with both viruses in BHK-21 cells at an MOI of 1, and no decline was observed with increasing MOI. Thus, virus protein synthesis in BSC-1 cells diminished with increasing MOI, suggesting that the apoptotic process itself interferes with virus production.

Effect of UV irradiation on TMEV-induced apoptosis. To determine whether TMEV induced apoptosis by signaling from the cell surface or intracellularly, UV-irradiated GDVII virions were adsorbed to BSC-1 cells. Although UV may induce in the picornavirion shell structure a conformational shift associated with extrusion of VP4 and loss of virion attachment to the cell receptor, 265-nm UV is absorbed 20 times more efficiently by nucleic acid than by protein, providing a margin of safety from damage to the shell structure (12, 30). Therefore, GDVII virus was inactivated for 1, 2, 4, 8, and 16 min with UV light, which resulted in a loss of at least 99% of the titer (not shown) without loss of binding to BSC-1 cells as demonstrated by flow cytometry (Fig. 6A). At MOIs of 10, 100, and 1,000, UV-irradiated virus failed to induce apoptosis compared with nonirradiated virus as measured by the MTT assay (Fig. 6B). Thus, TMEV-induced apoptosis in BSC-1 cells must be triggered by virus interaction with cytoplasmic proteins rather than with cell surface proteins.

DISCUSSION

In this study, both GDVII and BeAn viruses induced apoptosis in restrictive BSC-1 cells, as indicated by the characteristic features of (i) internucleosomal DNA degradation with fragmentation and margination of chromatin and (ii) cellular shrinkage. BSC-1 cell death was delayed until 10 to 12 h after infection and reached maximal levels by 20 to 24 h. Although only 0.5 to 1 PFU per BSC-1 cell was produced by infection with either TMEV strain, a substantial amount of cytoplasmic virus antigen was detected by flow cytometry, and the synthesis of virus capsid proteins was readily observed by [^{35}S]methionine labeling of these cells. Thus, although BSC-1 cells are infected, the infectious process appears to be severely limited, such that not every cell produces infectious virus. Apoptosis may further limit virus production. TMEV infection of permissive BHK-21 cells failed to trigger apoptosis, possibly because rapid cell death did not allow the apoptotic process to develop or because of lack of necessary proteins or proteases mediating cell death or inhibition of host protein synthesis under the permissive conditions of picornavirus replication. These findings are consistent with those of Tolskaya et al. (34), who demonstrated apoptosis in HeLa-S3 cells infected with mutant polioviruses under nonpermissive conditions (a guanidine-sensitive poliovirus mutant in the presence of guanidine, a guanidine-dependent poliovirus mutant in the absence of guanidine, and certain temperature-sensitive mutants at the restricted temperature), whereas wild-type poliovirus did not induce apoptosis in these cells. Vaccinia virus infection of nonpermissive CHO cells provides another example of abortive infection triggering apoptosis (10). In contrast, most other viruses reported to trigger apoptotic cell death do so in the context of productive infection (28).

The mechanism of cell death (and cell survival) and the regulated signaling pathways in virus-induced apoptosis are largely unknown. Several intracytoplasmic processes involved in apoptosis, e.g., protease activation, kinase activation, and oncogene activation and deactivation, have been described (reviewed in references 14a and 32). The fact that TMEV replicates in BSC-1 cells, although to a limited extent, and could possibly induce the apoptotic process intracellularly does not exclude a plasma membrane receptor signaling event that initiates this process. However, the finding that UV-irradiated and inactivated TMEV with unchanged binding characteristics prevents the induction of apoptosis suggests that apoptosis in BSC-1 cells is triggered by intracytoplasmic events.

The highly virulent GDVII virus has at least a 50-fold-greater capacity to induce apoptosis than the less virulent BeAn virus, suggesting that neurovirulence of TMEV strains may correlate with the efficiency of induction of apoptosis. Since both viruses were similarly restricted in their growth in BSC-1 cells, the difference in apoptosis is not due to a difference in viral replication. However, immunofluorescent staining (tunnel assay) of brain sections from mice infected intracerebrally with GDVII virus has revealed numerous apoptotic cells in the affected neocortex, whereas mice infected with BeAn virus have virtually no positive-staining cells (unpublished data). This finding suggests that GDVII virus kills some CNS cells by an apoptotic mechanism, although it is not known whether these apoptotic cells in the neocortex are infected. The triggering of apoptosis by an encephalitic virus may represent a way of amplifying the neuropathological process. In

contrast, the lower efficiency of BeAn virus in inducing apoptosis may enable a balance between cell survival and virus replication that leads to TMEV persistence in the CNS of mice.

In TMEV persistent infections, macrophages that infiltrate demyelinating lesions bear most of the virus antigen burden (17). TMEV replication has been demonstrated to be highly restricted in macrophages cultivated from normal mouse brain (16) and in several mouse macrophage cell lines (11) as well as in macrophages isolated directly from the CNS of mice with TMEV-induced demyelinating disease (3). TMEV also induces apoptosis in mouse macrophage cell lines (unpublished data). Others have shown that monocytes/macrophages undergo apoptosis (18); however, apoptosis can be prevented by cytokine treatment (18, 19) and by activation (23) and infection of macrophages with bacteria and parasites (22, 39). If in TMEV-induced demyelinating disease, infected macrophages are prevented from undergoing apoptosis, for example, as a normal response to a reduction in cytokines or growth factors, an inflammatory response that ordinarily would be self-limiting may continue, thereby perpetuating the infection and contributing to the demyelinating process.

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