Apoptosis induction by the Therien and vaccine RA27/3 strains of rubella virus causes depletion of oligodendrocytes from rat neural cell cultures

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The induction of cell death by the Therien strain of rubella virus (RVT), and the vaccine RA27/3 strain, was investigated in mixed glial cell cultures derived from the rat CNS. Cell death induction in Vero and rat glial cells by RVT and RA27/3 was dependent on virus replication. In both cell types and for both virus strains, cell death induction had the hallmarks of apoptosis, as detected by DNA laddering, terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling staining and Annexin V staining. For rat mixed glial cells, the depletion of oligodendrocytes was due to the induction of apoptosis for both virus strains. The induction of apoptosis in H358a cells, which carry a homozygous deletion of the p53 gene, indicated that a p53-independent pathway can be involved. The induction of cell death by RVT and RA27/3 in Vero and rat glial cells was associated with caspase-3 activity. It is concluded that rubella virus (RV) induces apoptosis in oligodendrocytes in rat glial cell cultures by a caspase-dependent pathway and that similar mechanisms occur for both the RVT laboratory strain and the vaccine RA27/3 strain. The tropism of both strains of RV for oligodendrocytes and the induction of apoptosis in such cells may have important implications for the mechanism of virus neuropathogenesis.

Introduction

Rubella virus (RV) is the only member of the genus Rubivirus of the family Togaviridae. It is a positive-stranded RNA virus and its only known natural host is man. Its multiplication strategy is similar to the alphaviruses Sindbis virus and Semliki Forest virus (SFV), which are also members of the family Togaviridae and have been studied more intensively.

While post-natally acquired rubella usually presents as only a mild syndrome (with joint involvement being the most common complication), a foetus infected via its pregnant mother can sustain birth defects (collectively termed congenital rubella syndrome or CRS). The majority of CRS cases involve the CNS (Atkins et al., 1995). It has been shown that one of the initial steps in RV-induced teratogenesis involves the interaction of the RV replicase (NSP90) with the retinoblastoma tumour suppressor protein (pRb), which possibly alters foetal cell growth (Atreya et al., 1998).

Sequelae of RV infection include three distinct neurological syndromes: a post-infectious encephalitis following acute infection, a spectrum of neurological manifestations following congenital infection and a rare neurodegenerative disorder, progressive rubella panencephalitis (PRP), which can follow either congenital or post-natal infection. Virus invasion and replication in the CNS has only been demonstrated definitively in CRS and appears to account for the majority of neurological lesions observed in this disease. Acute rubella encephalitis is a demyelinating disease designated as secondary encephalitis. PRP is characterized by a prolonged asymptomatic period followed by the onset of symptoms of neural deterioration during the second decade of life. Serious neurological dysfunction follows, ultimately leading to death (Chantler et al., 2001). The loss of myelin and oligodendrocytes in PRP suggests a degree of selective cell tropism for RV in the CNS (Chantler et al., 2001; Townsend et al., 1976). There are similarities in the neuropathology of PRP and multiple sclerosis (MS), a disease long suspected to be triggered by a virus infection in late childhood (Atkins et al., 2000).

The RV vaccine strain used currently in the USA and Europe is the live attenuated Wistar RA27/3 strain. This strain was attenuated by passaging in WI-38 human diploid cell cultures. Vaccine strains of RV appear to lack the teratogenic capacity of natural (wild-type) RV (Signore, 2001). However,
potential neurological complications associated with rubella vaccination have raised some concern. Cusi et al., (1999) reported peripheral neuropathy associated with anti-myelin basic protein antibodies in a woman vaccinated with RA27/3. There has also been a case of acute disseminated encephalomyelitis following rubella vaccination (Tsuru et al., 2000).

There are no reliable animal model systems for the study of clinically symptomatic RV infection. However, the virus does multiply in cultured mouse embryos (Hearde et al., 1986), in some human and monkey cell lines (notably Vero cells), in a rabbit cell line (RK13) and in neural cell cultures derived from neonatal rat brain (Atkins et al., 1991; Natale et al., 1993; Van Alstyne & Smyrinis, 1984). We have shown previously that multiplication of RV in rat mixed glial cell cultures results in a partial cytopathic effect and disintegration of oligodendrocytes and that pure neuron cultures are not infected (Atkins et al., 1991; Natale et al., 1993).

A selective tropism for oligodendrocytes in the CNS has been shown for the alphavirus SFV, which induces immune-mediated demyelination in mice and rats (Atkins et al., 1985, 1990b, 1999; Balluz et al., 1993). For SFV, cell death and depletion of oligodendrocytes in cell culture occur by induction of apoptosis (Glasgow et al., 1997), which is a function of the nonstructural region of the virus genome (Glasgow et al., 1998).

Apoptosis has also been implicated in the pathogenicity of RV (Pugachev & Frey, 1998; Duncan et al., 1999; Hofmann et al., 1999; Megyeri et al., 1999). The induction of apoptosis appears to be a function of the nonstructural region of the virus genome (Pugachev et al., 1997), although the capsid protein may also be involved (Duncan et al., 2000). Previous studies have shown that RV cytopathology is partially inhibited by caspase inhibitors and have concluded from this that RV-induced apoptosis is caspase dependent (Pugachev & Frey, 1998; Duncan et al., 1999). Both p53-dependent (Megyeri et al., 1999) and p53-independent (Hofmann et al., 1999) pathways have been implicated in RV-induced apoptosis.

In this study, we have analysed the mechanism of induction of cell death by the Therien strain of RV (RVT) and the RA27/3 vaccine strain. We show that for both virus strains, selective infection of oligodendrocytes occurs in mixed glial cell cultures and that the depletion of such cells is due to the induction of apoptosis. We postulate that this may be related to the mechanism of neuropathogenesis of RV, in particular its ability to induce CNS demyelination.

Methods

Statistical analysis. All experiments were performed at least three times and at least three measurements were made for each time-point. For cell counts, the total number of cells counted for each time-point was at least 100. The software package PRISM, version 2.01, was used for statistical analysis; unpaired t-tests were used for single time-points but whole data sets were compared using analysis of variance, with a significance level of $P = 0.05$.

Viruses and cells. RVT was grown and plaque assayed using Vero cells, as described previously (Atkins et al., 1991). The RA27/3 vaccine strain (Ervevax) was purchased from SmithKline Beecham in lyophilized vials and was used directly after reconstitution and plaque assay on Vero cells. For all infection experiments, virus inocula were adjusted to give an m.o.i. of 1 p.f.u. per cell. For virus growth curve experiments, cultures were washed three times with PBS after adsorption and, subsequently, following the collection of culture fluid at each time-point for virus assay, the culture fluid was replaced. RVT and RA27/3 working stocks were UV-irradiated to render the RNA incapable of replication (Lai & Joklik, 1973). Virus was decanted into Petri dishes, to a depth of 1 mm, and then placed 10 cm from an 8 W UV lamp for 10 min. Loss of infectivity was measured by plaque assay. NCI-H358a cells, a human lung carcinoma cell line with a homozygous p53 deletion (Takahashi et al., 1989), were grown as described previously (Glasgow et al., 1998; Murphy et al., 2000). HLE0 cells, a human promyelocytic leukaemia cell line (Zisterer et al., 2000), were a gift from Margaret McGee, Department of Biochemistry, Trinity College Dublin, Ireland. HLE0 cells were used as a control cell line in caspase fluorogenic assays. These suspension cells were grown in supplemented RPMI 1640 medium containing 20% foetal calf serum. Primary rat glial cells were prepared from neonatal rat brain, as described previously (Atkins et al., 1990b, 1991). They contain about 20–30% oligodendrocytes, which form a layer above the majority of cells in the culture, which are mainly astrocytes.

Cell viability assays. Total cell counts and viable cell counts were determined by the trypan blue exclusion assay. Cells were removed from flasks by trypsinization with trypsin–EDTA. Floating cells were centrifuged at 3000 g at 10 °C for 5 min and the supernatant discarded. Cells were resuspended in growth medium and 0.5 ml 0.4% (w/v) trypan blue solution, 0.3 ml growth medium and 0.2 ml cell suspension were mixed thoroughly and allowed to stand for 10 min. Viable (nonstained) and nonviable (blue) cells were counted using a haemocytometer every 24 h from 1 to 14 days post-infection (p.i.). This procedure was repeated in triplicate.

Immunocytochemistry. Cell cultures were plated in 4-well chamber slides at a density of 10° cells/ml. A panel of four fixatives was tested to determine the most suitable fixative for each cell type. The fixatives were: 10% neutral buffered formalin (NBF), methanol, 4% paraformaldehyde and acetone. Cells fixed in 10% NBF were post-fixed in ethanol–acetic acid (2:1) for 5 min at −20 °C. Endogenous peroxidase was quenched in 3% (v/v) H2O2 in PBS for 10 min. Cells were washed twice with PBS between each step. Immunocytochemistry was carried out with the Vectastain Elite ABC kit (Vector Laboratories), according to the manufacturer’s instructions but with the following exceptions: cells were incubated sequentially at 37 °C in a humidified chamber with normal serum for 20 min and primary antibody for 40–60 min. Primary antibodies were tested at varying dilutions in PBS to determine optimal staining. Slides were then incubated for 50 min with biotinylated secondary antibody and avidin–biotin–peroxidase complex for 30 min in a humidified chamber at room temperature. Between each step, slides were washed twice with TWEEN-80 (0.05% in PBS) and PBS. Peroxidase staining was localized with DAB (Sigma). Cells were incubated with DAB for 2 min and washed under running water for 10 min. Cells were counterstained in 2% methyl green in 0.1 M sodium acetate for 10 min and mounted in DPX-mounting medium. For immunofluorescent staining, cells were incubated sequentially with normal serum (from the same species as the secondary antibody, diluted 1:20 in PBS) for 1 h and primary antibody for 1 h at 37 °C in a humidified chamber. Slides were then incubated with either a FITC- or a TRITC-conjugated secondary antibody for 1 h at 37 °C. After addition of primary and secondary antibodies, cells were washed twice with PBS–TWEEN-80 and with PBS.
Primary and secondary antibodies were substituted with PBS to detect background and nonspecific staining. Primary antibodies utilized were mouse anti-2',3'-cyclic nucleotide 3'-phosphodiesterase (anti-CNPase, monoclonal; diluted 1:10 in PBS) (Sigma) for the detection of oligodendrocytes and rabbit anti-cow gial fibrillary-associated protein (anti-GFAP, polyclonal; diluted 1:100 in PBS) (Sigma) for the detection of astrocytes. For detection of RV antigen, a hyperimmune human serum from a vaccinated volunteer was used at a dilution of 1:1000; at this dilution, it produced bright fluorescence of infected cells compared to uninfected cells or normal human serum.

Detection of apoptosis. Detection of DNA fragmentation by laddering and terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) staining was carried out as described previously (Glasgow et al., 1997, 1998; Murphy et al., 2000). For Annexin V staining, cells were infected with virus, then rinsed once with binding buffer (Apoalert Annexin V kit) (Clontech), followed by incubation with 200 µ binding buffer, 5 µL Annexin V and 10 µL propidium iodide for 10 min in the dark at room temperature. Cells were fixed in 2% formaldehyde for 10 min. Fluorescence was detected with FITC and TRITC filters using a fluorescent microscope.

Caspase protease activity. Cytosolic extracts were prepared by the method of Zisterer et al. (2000). Cells were harvested by centrifuging at 3000 g for 5 min. Pellets were washed in 10 mL PBS and then resuspended in 200 µL harvesting buffer [20 mM HEPES pH 7.5, 10% (w/v) sucrose, 0.1% (w/v) CHAPS, 2 mM DTT, 0.1% (v/v) igepal, 1 mM EDTA, 1 mM PMSF in isopropanol, 1 µg/mL leupeptin and 1 µg/mL pepstatin]. Samples were left on ice for 10 min. Cells were lysed by being drawn through a 21-gauge needle eight times. Samples were left on ice for a further 5 min, followed by centrifuging at 15,000 r.p.m. for 10 min at 4 °C. The supernatant was either used fresh to measure caspase activity or stored at −20 °C. To determine caspase activity, cytosolic extract containing 10 µg protein was made up to 300 µL with incubation buffer [100 mM HEPES pH 7.5, 10% (w/v) sucrose, 0.1% (w/v) CHAPS and 10 mM DTT]. A sample of 1.2 µL 5 mM caspase-3 substrate, Ac-DEVD-AMC, was added to each sample to give a final concentration of 20 µM. Following incubation at 25 °C for 60 min, fluorescence was measured using a spectrofluorimeter (excitation wavelength 380 nm and emission wavelength 460 nm). The amount of 7-amino-4-methyl coumarin (AMC) released was determined by comparison with a standard curve generated with known amounts of AMC. UV-irradiated HL60 cells were used as positive controls for caspase activity. The specific activity of caspase-3 protease was calculated from the AMC released plotted against time.

Results

Cell death induction in Vero cells

In RVT-infected Vero cells, cytopathic effect (CPE) was first observed approximately 2–3 days p.i. By 6 days p.i., CPE was extensive and the majority of cells had lifted from the monolayer. However, with RA27/3, CPE appeared more slowly and was partial, initially being observed at approximately 4–5 days p.i. Cells became infected and rounded-up but only a small percentage lifted from the monolayer. The titres of infectious virus were determined from 1 to 14 days p.i. for Vero cells infected with RVT and RA27/3 (Fig. 1A). For RVT-infected cells, virus titres peaked at 4 days p.i. In comparison, virus titres for RA27/3 were about 3 logs lower but peaked at the same time. Trypan blue exclusion assays were conducted to compare the differences, in cell viability and cell counts, between Vero cells infected with RVT, RA27/3 and mock-infected. Data were collected over 14 days for monolayer and floating cells (Table 1). Prior to infection, cell viability of healthy subconfluent cells was 80–90%. RVT-infected cells displayed a decrease in cell viability, which, by 14 days p.i., was 10 times lower than control cells. For RA27/3, the decrease, although not as marked as for RVT, was still significant compared to mock-infected control cells. The differences between RVT- and RA27/3-infected cells were significant.

The induction of apoptosis in Vero cells was confirmed by the detection of DNA ladders by agarose gel electrophoresis (Fig. 1B). DNA was extracted from infected and control cells from 1 to 14 days p.i. DNA ladders were detected from 6 to 14 days p.i. in RVT-infected cells. The most intense DNA ladders occurred between 7 and 10 days p.i. For RA27/3, less intense DNA ladders were observed from 8 to 14 days p.i. No DNA ladders were detected in mock-infected control cells (up to 20 days p.i.).
**Table 1. Staining of cells for viability, apoptosis and viral antigen**

Data are presented for floating and monolayer cells combined. All controls were mock-infected and indicate background staining. Probabilities quoted are from analysis of the total data from all time-points.

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* Probability of RVT and RA27/3 compared.
† Percentage of unstained cells.

TUNEL-positive Vero cells, indicative of the induction of apoptosis (Fig. 2A, B), were quantified and the percentage of apoptotic cells compared. Monolayer and floating cells were quantified separately. For uninfected monolayers, there was a low level of TUNEL-positive cells. The differences between control and virus (RVT and RA27/3)-infected cells were significant (Table 1). There was no significant difference between RVT- and RA27/3-infected monolayers over the 14-day period. All floating cells were TUNEL-positive for RVT-, RA27/3- and mock-infected cells. The number of floating cells was hence quantified and added to the number of TUNEL-positive monolayer cells to give the overall percentage of TUNEL-positive cells (Table 1). When the total data (monolayer and floating cells) was analysed, the differences between control and infected cells, and between RVT- and RA27/3-infected cells, were significant. The induction of apoptosis in Vero cells was confirmed by Annexin V staining; this detects the translocation of phosphatidyl serine from the inner to the outer surface of the cell membrane during the early stages of apoptosis. The onset of apoptosis was detected as early as 18 and 24 h p.i. for RVT- and RA27/3-infected cells, respectively (Fig. 2C, D). Only 10–20% of Annexin V-stained cells contained propidium iodide, indicating that most cells were apoptotic rather than necrotic (data not shown).

The number of cells expressing viral antigen was quantified in cells infected with RVT or RA27/3 or mock-infected (Fig. 2E, F) from 1 to 14 days p.i. for monolayer and floating cells. All floating cells from infected cultures were positive for viral antigen from 1 to 14 days p.i. The number of positive floating cells was quantified and combined with the number of positive monolayer cells to give the total percentage of cells positive for viral antigen (Table 1). For the combined results, the number of positive cells in RVT-infected cultures reached 98% by 14 days p.i. In comparison, the percentage for RA27/3-
Fig. 2. Examples of staining for apoptotic cells and viral antigen. Original magnification in all cases × 400. (A, B) TUNEL staining of Vero cells (brown) at 7 days p.i. (A) Mock-infected cells. (B) Cells infected with RA27/3. (C, D) FITC-conjugated Annexin V-labelled rat glial cells at 98 h p.i. (C) Mock-infected cells at 96 h p.i. An occasional cell shows staining. (D) RA27/3-infected cells. (E, F) Staining of Vero monolayer cells with human anti-RA27/3 antisera (brown). (E) Mock-infected cells at 8 days p.i. (F) RVT-infected cells at 8 days p.i. showing CPE. (G, H) Immunofluorescent labelling of oligodendrocytes. Rat mixed glial cells were infected or mock-infected and labelled with anti-CNPase at 10 days p.i. (G) Mock-infected cells. (H) RA27/3-infected cells showing rounded apoptotic morphology. (I, J) Immunofluorescent labelling of astrocytes. Rat mixed glial cells were infected or mock-infected with RVT or RA27/3 and labelled with anti-GFAP at 10 days p.i. (I) Mock-infected cells. (J) RVT-infected cells showing no morphological change. (K, L) Dual fluorescent labelling of infected apoptotic rat glial cells (oligodendrocytes). Cells were infected with RA27/3 and double immunofluorescently labelled at 10 days p.i. (K) TUNEL-positive (FITC) RA27/3-infected cells. (L) The same cells stained for viral antigen (TRITC). Note the blebbing characteristic of apoptosis.
infected cells was lower and there was a significant difference in the number of antigen-positive cells between the two virus strains. Dual-labelled Vero cells were examined by fluorescence microscopy by FITC- and TRITC-labelling. Virus-positive (TRITC) and TUNEL-positive (FITC) cells did coincide; however, for RVT- and RA27/3-infected cells (7 days p.i.), a larger proportion of cells was positive for viral antigen than for TUNEL staining (data not shown).

**Cell death induction in H358a cells**

CPE was first observed in both RVT- and RA27/3-infected H358a cells at approximately 3 or 4 days p.i. This CPE was partial, with only a small percentage of infected cells floating in the supernatant. Virus titres peaked at 8 days p.i. but there was no significant difference between the virus titres for RVT and RA27/3 (Fig. 3A). As with Vero cells, data were collected for monolayer and floating cells, over 14 days, to compare the differences in cell viability between cells infected with RVT and RA27/3 and uninfected controls. Prior to infection, cell viability of healthy confluent cells was approximately 85–95%. There was a significant difference in the percentage cell viability between infected and uninfected H358a cells but no significant difference between the two virus strains (Table 1). DNA ladders were detected in RVT-infected H358a cells from 4 to 14 days p.i. The most intense DNA ladders occurred at 5 days p.i. For RA27/3-infected H358a cells, DNA ladders were detected from 5 to 14 days p.i.; RA27/3-induced DNA fragmentation was not as intense as for RVT (Fig. 3C). The increase in TUNEL-positive H358a cells when infected with RVT or RA27/3 was significant but there was no significant difference between the two virus strains (Table 1). The induction of apoptosis in H358a cells by RVT and RA27/3 was further confirmed by Annexin V staining (data not shown). Apoptotic cells were observed as early as 3 days p.i. for both virus strains. Viral antigen was detected in H358a cells infected with RVT and RA27/3 but there was no significant difference between RVT and RA27/3 infected cells (Table 1).

**Cell death induction in rat mixed glial cell cultures**

CPE induced by RVT in infected rat glial cells was slow in appearance and only partial, as has been reported previously (Atkins et al., 1991; Natale et al., 1993). Infection with RA27/3 also resulted in slow and partial CPE. Virus titres in RVT- and RA27/3-infected rat glial cells were low, peaking at 10 days p.i. (Fig. 3B). There was no significant difference between virus strains. That virus multiplication was indeed occurring in the
cultures despite the low virus titres was confirmed by detection of infected cells by viral antigen labelling (see below).

Data were collected for monolayer and floating cells (separately and combined), over 14 days, to compare the differences in cell viability between rat glial cells infected with RVT and RA27/3 and uninfected controls (Table 1). There was no difference in total cell viability between control and RVT-infected cells until 5 days p.i., when virus infection resulted in a drop in cell viability. There was no significant difference between RVT- and RA27/3-infected cultures.

The induction of apoptosis in rat glial cells was confirmed by the detection of DNA fragmentation by agarose gel electrophoresis. DNA ladders were detected from 7 to 14 days p.i. in RVT-infected cells. For RA27/3, DNA ladders were observed from 9 or 10 days p.i. to 14 days p.i., although their appearance was not as definitive as for RVT (Fig. 3D).

As observed for Vero and H358a cells, rat glial cells stained brownish–black by TUNEL staining. Nonapoptotic cells were counterstained with methyl green. The TUNEL-positive nuclei in infected rat glial cells exhibited morphology characteristic of apoptosis, with condensation of nuclear chromatin and/or nuclear fragmentation. All floating cells were TUNEL-positive for RVT-, RA27/3- and mock-infected rat glial cells from 1 to 14 days p.i. The number of floating cells was hence quantified and added to the number of TUNEL-positive monolayer cells to give the overall percentage of TUNEL-positive cells. When the total data (monolayer and floating cells) was analysed, there was a significant increase in the percentage of TUNEL-positive cells for RVT-infected cells and this increase was mirrored for RA27/3-infected cells. There was no significant difference between RVT and RA27/3. Annexin V staining detected phosphatidyl serine on the cell surface of apoptotic rat glial cells as early as 72–96 h p.i. for both RVT- and RA27/3-infected cells (data not shown).

For viral antigen staining, data were analysed for monolayer and floating cells separately and combined. When the complete dataset was analysed, there was no significant difference between RVT- and RA27/3-infected cells, although there was a clear difference between infected and control cells (Table 1).

Immunofluorescent labelling of oligodendrocytes and astrocytes

Infection of rat glial cells with RVT or RA27/3 resulted in the depletion of the upper oligodendrocyte layer (Fig. 2G, H), leaving the lower astrocytic layer intact. In infected cultures, oligodendrocytes rounded-up and were reduced in number (Fig. 2H). Oligodendrocytes in uninfected cultures did not undergo any morphological changes. In contrast, there was no morphological difference in astrocytes, between infected and uninfected cultures, nor were astrocytic cells reduced in number when infected with the wild-type or vaccine strains of RV (Fig. 2I, J). TUNEL staining colocalized with cells positive for viral antigen and for oligodendrocytes (Fig. 2K, L).

Role of virus replication in RV-induced apoptosis

No DNA ladders were observed in Vero or rat glial cells infected with UV-inactivated RVT or RA27/3 up to 14 days p.i. DNA ladders were observed in positive controls (cells infected with RVT and RA27/3); this was confirmed by TUNEL staining. Similar results were obtained for supernatants depleted of virus by centrifugation.

Analysis of caspase activity

For caspase-3, there was a significant difference in specific activity between infected Vero, rat glial and control cells (Fig. 4). Treatment of both Vero and glial cells with the caspase inhibitor z-DEVD-fmk restored cell viability to control levels following infection with both strains of RV (data not shown).

Discussion

In this study, we have shown that both the laboratory Therien and vaccine RA27/3 strains of RV induced apoptosis in Vero cells. The hallmarks of apoptosis were examined, including internucleosomal DNA fragmentation (DNA ladders and TUNEL staining), translocation of phosphatidyl serine (Annexin V staining) and caspase-3 induction. This has confirmed the results of other workers using different cell lines, virus strains and m.o.i. (Pugachev & Frey, 1998; Hofmann et al., 1999; Megyeri et al., 1999; Duncan et al., 1999, 2000), although this is the first time that the RA27/3 vaccine strain.

![Fig. 4. Caspase-3 activity in infected cells. Cells were infected with either RVT or RA27/3 and caspase-3 specific activity was measured from 1 to 14 days p.i. using fluorometric assays. Control cells were mock-infected. (A) Caspase-3 protease specific activity in Vero cells. (B) Caspase-3 protease specific activity in rat glial cells.](Image 336x600 to 531x716)
has been included in such a study. It is also the first time that apoptosis induction by RV has been detected in primary neural cells. A p53-independent pathway is involved, confirming a previous report (Hofmann et al., 1999).

The lack of detectable apoptosis in cultures infected with UV-inactivated RV suggested that productive virus infection is necessary to trigger cell death. For other togaviruses, it has been shown that virus replication is required for apoptosis induced by SFV (Glasgow et al., 1998) but not required for Sindbis virus (Jan & Griffin, 1999). The inability of virus-depleted medium to induce apoptosis indicates that no other component of the virus inoculum is involved in apoptosis induction.

In order to establish whether apoptosis induction by RV requires p53, the H358a human lung carcinoma cell line was used, which carries a homozygous p53 deletion. The results of this study indicate that p53-independent apoptosis can be induced by RV. Although the proportion of infected cells undergoing apoptosis in this cell line was less than for Vero cells, this was correlated with a correspondingly lower proportion of cells expressing viral antigen. These results are consistent with a previous study by Hofmann et al. (1999), where no significant changes in the levels of expression of p53 were observed following acute or persistent RV infection in Vero cells. Apoptosis independent of p53 can also be induced by SFV (Glasgow et al., 1998; Murphy et al., 2000). Our results do not preclude the possibility that p53-dependent apoptosis may also be induced by RV, as described by Megyeri et al. (1999).

This study established that the depletion of the upper oligodendrocyte layer, described previously for rat glial cell cultures after RVT infection (Atkins et al., 1991; Natale et al., 1993), is due to apoptotic cell death. It has also demonstrated that the live attenuated vaccine strain RA27/3 induces apoptosis in these cells. It has been shown previously that SFV induces apoptotic cell death of oligodendrocytes in rat glial cell cultures (Glasgow et al., 1997). The occurrence and extent of apoptosis was quantified for both RV strains and there was no significant difference between the two. The only difference was that DNA ladders were observed 1–2 days earlier in RVT- than in RA27/3-infected cells. Similarly, when rat glial cells were quantified for the presence of viral antigen, there was no significant difference between RVT and RA27/3 and both infected oligodendrocytes.

RV induces two diseases of the CNS, post-infectious encephalitis and PRP, both of which may involve autoimmune demyelination. In addition, RV has been implicated as a trigger for MS (along with several other viruses), a much more common disease (Atkins et al., 2000). In two rare demyelinating diseases of the CNS known to be associated with persistent virus infection, subacute sclerosing panencephalitis (associated with measles virus) and progressive multifocal leukoencephalopathy (associated with JC virus), infection of oligodendrocytes has been demonstrated (Atkins et al., 2000). Two mechanisms have been suggested as possible initiators of RV-induced demyelination. One is molecular mimicry, where sequence similarity between viral and myelin proteins could trigger anti-myelin autoimmunity. Several sequence similarities have been described between RV and myelin proteins (Atkins et al., 1990a; Cusi et al., 1999; Besson Duvanel et al., 2001). Another possibility is that infection of oligodendrocytes could lead to the release of normally sequestered myelin antigens and their presentation to the immune system, thus generating primary demyelination which subsequently leads to anti-myelin autoimmunity (Atkins et al., 1994, 2000). In this and previous studies (Atkins et al., 1991; Natale et al., 1993), we have shown that RV has a tropism for oligodendrocytes in rat glial cell cultures and induces apoptosis in such cells. In this respect it is similar to SFV, which induces autoimmune demyelination in mice (Atkins et al., 1999). It is not known whether this occurs in human cells, but if such a mechanism operates, it could lead to virus-induced demyelination by both the wild-type and vaccine strains.

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References


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