Suppression of apoptotic and necrotic cell death by poliovirus

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To determine an antiapoptotic activity of poliovirus type 1 (PV-1), we examined the effect of PV-1 infection on apoptosis that was induced in HEp-2 cells by the treatment with 1 M sorbitol. The virus did not induce apoptosis in the infected cells and could suppress both the fragmentation of chromosomal DNA and morphological cell and cell nuclei changes in the sorbitol-treated cells, indicating that PV-1 induces an antiapoptotic state. Comparison of the kinetics showed that this ability of the virus appeared in the infected cells at the time of progeny virus formation (maturation step of virus multiplication). Simultaneously with this antiapoptotic activity, PV-1 infection also suppressed non-apoptotic cell death induced by sodium chloride. Electron microscopic observation revealed that the cells killed by the sodium chloride treatment had undergone liquefactive necrosis, indicating that PV-1 can inhibit both apoptosis and necrosis. In addition, PV-1 can grow in the apoptotic cells, although the virus yield was reduced to a quarter of the yield in normal cells.

Introduction

Although many animal viruses can induce apoptosis in infected cells, large DNA viruses, such as poxviruses, herpesviruses and adenoviruses, usually exhibit the ability to suppress the induction of apoptosis in the infected cells (for review see Koyama et al., 1998a; Roulston et al., 1999; Shen & Shenk, 1995). Previously we found that the treatment of HEp-2 cells with sorbitol rapidly induces massive apoptosis and provides a unique, highly efficient and decisive method to detect the ability of the virus to suppress apoptosis in infected cells (Koyama & Miwa, 1997; Koyama et al., 2000a). Although treatment to induce apoptosis often requires the inhibition of macromolecular synthesis in the treated cells, the sorbitol treatment does not require such inhibition, allowing virus multiplication in the treated cells. With this method, we found antiapoptotic function in herpes simplex virus (HSV) type 1 (Koyama & Miwa, 1997) and type 2 (Hata et al., 1999) and human immunodeficiency virus (HIV) (Fukumori et al., 1998).

In contrast to these DNA viruses, RNA viruses in general are thought not to carry an antiapoptosis gene. Under permissive conditions, they usually induce apoptosis in the infected cells but escape a deleterious effect of apoptosis by rapid multiplication (Koyama et al., 1995, 1998c; Kurokawa et al., 1999; for review see Koyama et al., 1998a). The only exception among these orthodox RNA viruses is poliovirus (PV) (HIV is the other exception among RNA viruses; Fukumori et al., 1998). Tolskaya et al. (1995) reported that, although PV induces apoptosis of the infected HeLa cells under non-permissive conditions for virus multiplication, the virus does not induce apoptosis in the productive infection and, by characterizing the effects of PV infection on apoptosis induced by the inhibitors of protein synthesis or RNA synthesis, they concluded that PV has an antiapoptosis function. However, the use of those inhibitors (1) blocks the progress of the normal virus infection process and (2) takes longer (approximately 4–5 h by actinomycin D) to induce apoptosis than sorbitol-induced apoptosis (within 2 h). In addition, we previously demonstrated that Sendai virus does not induce apoptosis in most of the infected cells, but this virus does not have an antiapoptosis gene, indicating that the lack of apoptosis in the virus-infected cells is not always accompanied by the presence of an antiapoptosis gene in the virus (Koyama et al., 2001). Furthermore, PV-induced apoptosis has been observed even under permissive conditions: in a mouse central nerve system (Girard et al., 1999), in a human enterocyte-like cell line (Ammendolia et al., 1999) and in a human promonocytic cell line (Lopez-Guerrero et al., 2000). These observations are inconsistent with the presence of an antiapoptosis gene in the PV genome.

To elucidate the nature of PV infection in regard to virus...
ability to regulate cell death, we examined apoptosis and an antiapoptotic function in the PV-infected cells by our highly definitive system described above (Koyama & Miwa, 1997; Koyama et al., 2000a). In addition, we also examined the effect of the virus infection on non-apoptotic necrosis-like death. Necrosis has been defined as another one of two types of eukaryotic cell death (i.e. apoptosis and necrosis), but the characteristics of necrosis are still much less clear than those of apoptosis. Previously, we found that the treatment of HEp-2 with sodium chloride induces death of the treated cells without the characteristics of apoptosis (Koyama et al., 2000a). Considering that, regardless of the types of cell death, death of the infected cells can bring interruption of virus multiplication, we characterized cell death induced by the sodium chloride treatment and examined the ability of PV to block non-apoptotic death.

Methods

Cells and virus. HEp-2 cells were used throughout the experiments. Cells were grown in Eagle’s minimum essential medium (MEM) supplemented with 5% foetal bovine serum (FBS). Poliovirus type 1, Sabin strain, (PV-1) was used throughout the experiments. Virus infection was carried out with essentially the same procedure as that described previously (Koyama & Uchida, 1994). To determine the virus growth curve, HEp-2 cells were infected with the virus at an m.o.i. of 10 and incubated at 35.5 °C in MEM containing 0.5% FBS. At the indicated times, the cell lysates were harvested after three cycles of freezing and thawing of the infected cells along with medium. The numbers of infectious virus in the lysates were determined by a plaque assay and represented total progeny viruses. For the determination of the amount of cell-free virus, the number of infectious virus particles in the culture medium was determined.

Plaque assay of PV-1 was carried out as follows. Virus samples were diluted with PBS containing 0.1% BSA and added to the confluent monolayers of Vero cells. The infected monolayers were incubated at 35-37 °C in MEM containing 0.5% FBS and 0.6% methy cellulose. After 2 or 3 days of incubation, the infected cells were fixed and stained simultaneously with 0.5% crystal violet solution containing 10% formalin.

Induction of apoptosis and necrosis. To induce apoptosis, confluent monolayers of HEp-2 cells were incubated at 37 °C in MEM containing 10 M sorbitol for 60 min. The treated cells were washed once with prewarmed MEM and further incubated at 37 °C in MEM for 60 min (Koyama et al., 2000a). For the induction of necrosis, 0.5 M sodium chloride was used instead of sorbitol (Koyama et al., 2000a).

Detection of chromosomal DNA fragmentation. Fragmentation of chromosomal DNA was determined by the method described previously (Koyama et al., 1995; Koyama & Miwa, 1997). Briefly, monolayered cells were harvested with trypsin, and fragmented DNA was extracted from the cells by the Hirt method (Hirt, 1967) with minor modifications and analysed for oligonucleosomal DNA ladders by electrophoresis on a 1.5% agarose gel.

For quantification of the chromosomal DNA extracted in the fragmented DNA fraction, chromosomal DNA was metabolically labelled with [3H]thymidine prior to infection and the radioactivity in fractions of the total and extracted DNA was determined separately. Relative amounts of the fragmented DNA were calculated by a ratio of radioactivity of the extracted DNA to that of the total cellular DNA.

Observation of nuclear morphology. Morphology of the infected cell nuclei was examined after the cells were fixed and stained with DNA-binding dye Hoechst 33258 (0.05 μg/ml) according to the method used by McGarrity (1979).

Electron microscopy. The examination was performed by the pop-off method (Yasuda & Toida, 1986). Briefly, the cells, cultured on glass slides, were fixed by immersing the slides in 2% glutaraldehyde solution for 30 min and post-fixation was done with 1% OsO4 solution for 1 h in a glass container. Then, cells on the slides were washed with buffer solution and dehydrated with graded ethanol. Epon monomer, mixed with hardener and accelerator, was prepared in O capsules and the capsules were placed upside down over the specimens on the glass slides. Polymerization was carried out at 60 °C for 4 days. After the polymerization was completed, careful trimming and ultrathin sectioning of the block were done, followed by staining with uranyl acetate and lead citrate. The specimens were observed under the electron microscope (JEM, 1200EX).

Results

Lack of apoptosis in the PV-infected cells

PV-1 grows rapidly in the infected cells. Fig. 1 shows the one-step growth curve of the virus in HEp-2 cells; the progeny virus appeared at 4 h post-infection (p.i.), increased with time and reached a plateau at 7 h.p.i. Cell-free virus appeared in the medium at 8 h.p.i. and increased with time with a significant delay to the formation of intracellular progeny viruses. Cytopathic effect of the virus infection could be found during the middle stage of the infection (4 or 5 h.p.i.) and became notable at 6 h.p.i., followed by an extensive rounding and ballooning of the infected cells at the later stage of infection (9 h.p.i. or later).

Previously we have shown that a productive infection with some RNA viruses, such as vesicular stomatitis virus (Koyama, 1995; Koyama et al., 1998c) or influenza virus (Kurokawa et al., 1999), induces massive apoptosis in the infected cells. Two classical signs of apoptosis, a cleavage of chromosomal DNA into oligonucleosome-sized fragments and a characteristic morphology of cells and cell nuclei (Kerr & Harmon, 1991), are observed clearly in the cells infected with these viruses. In contrast to these viruses, the fragmentation of chromosomal DNA was not observed at any stage of the PV-1 infection in HEp-2 cells (first five lanes from the left in Fig. 2). And, as shown in the later section of this report, cell nuclei with apoptotic morphology were not observed in the PV-1-infected cells. These results indicate the lack of apoptosis at any stage of infection in the PV-1-infected cells, confirming the previous conclusion of Agol and his coworkers (Tolkskaya et al., 1995).

Suppression by PV-1 of sorbitol-induced apoptosis and of sodium chloride-induced non-apoptotic cell death

To detect antiapoptotic ability of PV-1, we examined the effect of PV-1 infection on sorbitol-induced apoptosis. Temporal incubation of HEp-2 cells in the medium containing 1 M sorbitol induces extensive apoptosis in the treated cells within
60 min of incubation in the sorbitol-free medium (Koyama & Miwa, 1997; Koyama et al., 2000a). The commitment to cell death took place in most cells during the first 40 min of the incubation with sorbitol (Koyama et al., 2000a). When HEP-2 cells were infected with PV-1 prior to the sorbitol-treatment (middle five lanes in Fig. 2), the fragmentation of the chromosomal DNA into characteristic oligonucleosomal DNA was observed in the infected cells treated with sorbitol at 0 or 2 h p.i. However, at 4 h p.i., the fragmentation was slightly affected in the cells and was markedly suppressed in the cells treated with sorbitol at 6 or 8 h p.i., indicating that the induction of apoptosis is suppressed by the PV-1 infection at a late stage of virus multiplication.

Fig. 2 also shows the effect of PV-1 infection on the DNA degradation induced by sodium chloride. As reported previously (Koyama et al., 2000a), the temporal incubation of HEP-2 cells in the medium containing 0.5 M sodium chloride, with the osmotic pressure equivalent to 1 M sorbitol, induces a massive cell death without the apoptotic characteristics. Extensive degradation of chromosomal DNA occurs in the treated cells within 60 min of incubation in the reagent-free medium, but only a smear of DNA, products of a non-specific degradation of chromosomal DNA, is observed by agarose gel electrophoresis (Koyama et al., 2000a). Not only do they lack a DNA ladder, the sodium chloride-treated cells show a nuclear morphology quite different from that of the apoptotic cells as shown in the latter section. These characteristics of the dead cells after sodium chloride treatment indicate that this death is not apoptosis. The nature of sodium chloride-induced cell death will be described in the latter section of this report.

As shown in the last five lanes from the left in Fig. 2, the infection with PV-1 also suppressed the sodium chloride-induced degradation of chromosomal DNA. When the infected cells were treated with sodium chloride at various times after the infection, a massive DNA smear, instead of the apoptotic ladder of the sorbitol-treated cells, was clearly observed in the cells treated with the reagent at 0 or 2 h p.i. However, this degradation was also noticeably affected by the treatment at 4 h p.i. and almost completely inhibited at 6 or 8 h p.i. These results indicate that the infection with PV-1 can suppress not only apoptotic DNA fragmentation induced by sorbitol but also non-apoptotic DNA degradation induced by sodium chloride.

Quantification of the fragmented DNA revealed the kinetics of virus ability to suppress the degradation of chromosomal DNA in the cells treated with sorbitol or sodium chloride (Fig. 3). Suppression of the sorbitol-induced fragmentation of chromosomal DNA could be detected in the PV-1-infected cells at 4 h p.i. and enhanced with time, reaching a

Fig. 1. One-step growth curve of PV-1 in HEP-2 cells. Confluent monolayers of HEP-2 cells were infected with PV-1 at an m.o.i. of 10. The infected cells were incubated at 35.5 °C in MEM containing 5% FBS. At the indicated time, the numbers of cell-free (∆) and total viruses (○) were assayed separately as described in the text.

Fig. 2. Fragmentation of chromosomal DNA in the PV-1-infected HEP-2 cells. HEP-2 cells were infected with PV-1 at an m.o.i. of 10. The infected cells were incubated at 35.5 °C in MEM containing 0-1% BSA. At the indicated time, the infected cells were mock-treated or treated with 1 M sorbitol or 0.5 M sodium chloride for 60 min, followed by incubation in the reagent-free medium for 60 min. The treated cells were harvested and fragmented DNA was extracted by the Hirt method (Hirt, 1967). An oligonucleosomal ladder was detected in a 1.5% agarose gel.

Fig. 3. Effect of PV-1 infection on the degradation of chromosomal DNA induced by sorbitol or sodium chloride. [3H]Thymidine-labelled HEP-2 cells were mock-infected (■) or infected with PV-1 (○, △, □) at an m.o.i. of 10. The infected cells were incubated at 35.5 °C in MEM containing 0-1% BSA. At the indicated time, the infected cells were mock-treated (■) or treated with 1 M sorbitol (○) or 0.5 M sodium chloride (△) for 60 min, followed by incubation in the reagent-free medium for 60 min. The treated cells were harvested and fragmented DNA was extracted. Relative amounts of fragmented DNA to total cellular DNA were determined as described in the text.
plateau level at 6 h p.i. Almost the same kinetics were observed in the expression of activity to suppress the sodium chloride-induced DNA degradation in the infected cells, although the suppression was more notable than that induced by sorbitol. The similarity of kinetics suggests that a single virus-coded function might be responsible for the blockage of both apoptotic and non-apoptotic responses in the reagent-treated cells.

**Morphology of the infected cells and cell nuclei**

Fig. 4 shows the morphology of the infected HEp-2 cells treated with sorbitol or sodium chloride. Cell shrinkage and cytoplasmic blebbing are the representative morphological characteristics of apoptotic cells (Kerr & Harmon, 1991). In agreement with the results of the fragmentation of chromosomal DNA (Figs 2 and 3), the infected cells which were treated with sorbitol immediately after the infection with PV-1 contained large numbers of shrunken cells with extensive cytoplasmic blebbing (Fig. 4B). On the other hand, most cells, treated with sodium chloride instead of sorbitol, were not shrunken but rounded without obvious cytoplasmic blebbing, although the sodium chloride-treated cultures contained similar numbers of damaged cells to the sorbitol-treated culture (Fig. 4C). At 4 h p.i., the PV-infected cells did not show clear cytopathic effect (Fig. 4D). However, when the cells were treated with sorbitol at 4 h p.i., the number of shrunken cells was significantly decreased (Fig. 4F) in comparison to the mock-infected cells (Fig. 4E). Similar results were obtained when the infected and mock-infected cells were treated with sodium chloride (Fig 4H and G, respectively), agreeing with the results of the kinetic studies on the expression of virus ability to suppress the reagent-induced DNA degradation (Fig. 3).

To further confirm the abilities of PV-1 to suppress sorbitol-induced apoptosis and sodium chloride-induced non-apoptotic death, we examined the morphology of the infected cell nuclei after treatment with these reagents. Even in the absence of the treatment, the infected cell nuclei started shrinking along with the progress of virus multiplication; more than half the cell nuclei had shrunk at 4 h p.i. (Fig. 5B) and the majority were shrunken and deformed at 6 h p.i. (Fig. 5C). When the cells were treated with sorbitol immediately after the infection with PV-1, the majority of the infected cell nuclei showed perinuclear condensation and fragmentation of chromatin, which are the representative signs of apoptotic cell nuclei (Fig. 5D). However, when the infected cells were treated with
sorbitol at later stages of the infection, the number of apoptotic nuclei was decreased at 4 h p.i. (Fig. 5E) and was hardly observed at 6 h p.i. (Fig. 5F).

In agreement with the lack of oligonucleosomal fragmentation (laddering) of chromosomal DNA (Fig. 2), HEp-2 cells, treated with sodium chloride immediately after the infection, showed a very unique morphology of cell nuclei, quite different from that of the apoptotic cell nuclei. The nuclei were markedly shrunken and ill-shaped, but chromatin was distributed evenly in the nuclei as small dots (Fig. 5G). In addition, these morphological alterations of the infected cell nuclei by sodium chloride were also inhibited by PV infection with the progress of virus multiplication (Fig. 5H, I), with kinetics similar to the suppression of the sorbitol-induced morphological changes.

Electron microscopic examination of the cells treated with sodium chloride

To further examine the nature of sodium chloride-induced cell death, we examined the reagent-treated cells with an electron microscope. Fig. 6(a, d) shows morphology of the mock-treated control HEp-2 cells. The cells had a clear nuclear structure, filled cytoplasm and a smooth cell surface membrane. When the cells were incubated in the medium containing sodium chloride for 60 min, most cells showed a severe disorganization of the cell structure even immediately after the incubation (Fig. 6b). After 60 min of an additional incubation in the reagent-free medium, many of the cells showed marked generalized swelling (swollen mitochondria, unrecognizable Golgi apparatus etc.), condensation of chromatin and disruption of the plasma membrane (Fig. 6c). These morphological characteristics of the cell injuries are consistent with those of liquefactive necrosis (Goudie, 1985). In contrast, the sorbitol-treated cells displayed a mild disorganization of the cell structure immediately after incubation with the reagent (Fig. 6e), but, after the incubation in the reagent-free medium, the cells showed an extensive surface blebbing, nuclear fragmentation and the formation of many membrane-bounded apoptotic bodies which are composed of cytoplasm and packed organelles with or without nuclear fragments (Fig. 6f).

Fig. 5. Morphology of the cell nuclei of the PV-1-infected cells treated with sorbitol or sodium chloride. HEp-2 cells were infected with PV-1 at an m.o.i. of 10. Immediately after the infection (A, D and G) and at 4 h p.i. (B, E and H) or 6 h p.i. (C, F and I), the infected cells were mock-treated (A–C) or treated with 1 M sorbitol (D–F) or 0.5 M sodium chloride (G–I) for 60 min, followed by incubation in the reagent-free medium for 60 min. The treated cells were fixed and stained with H33258 as described in the text.
Fig. 6. Electron microscopy of HEp-2 cells treated with sodium chloride or sorbitol. Confluent monolayers of HEp-2 cells were mock-treated (a and d) or treated with 0.5 M sodium chloride (b and c) or 1 M sorbitol (e and f) for 60 min (a, b and e), followed by incubation in the reagent-free medium for 60 min (c, d and f). The treated cells were fixed, stained and observed as described in the text. Original magnification, ×1200 (b), ×1500 (e), ×2000 (a, c and d) and ×2500 (f).

Fig. 7. One-step growth curve of PV-1 in the apoptotic cells. Confluent monolayers of HEp-2 cells were incubated at 37 °C in MEM supplemented with (●) or without (○) 1 M sorbitol for 60 min. The cells were washed once with MEM and infected with PV-1 at an m.o.i. of 10. The infected cells were incubated at 35–35 °C in MEM. At the indicated time, the numbers of total progeny viruses were determined as described in the text.

The observed characteristics show the typical morphology of apoptotic cells (Kerr & Harmon, 1991). These results indicate that death by sodium chloride is necrosis while death by sorbitol is apoptosis.

Multiplication of PV in the apoptotic cells

Since the findings that apoptosis induced by insect virus causes premature death of the infected cells and aborts virus multiplication in vivo and in vitro, virus-induced apoptosis has been considered to be one of the host non-specific defence mechanisms against virus invasion (for review see Koyama et al., 1998a). We examined the effect of apoptosis on the multiplication of PV by infecting sorbitol-induced apoptotic cells with PV-1. As shown in Fig. 7, although the yield of progeny virus was significantly decreased even at 24 h p.i. (approximately a quarter of that in the control mock-treated cells), PV-1 can grow significantly in the apoptotic cells without any delay in the formation of progeny virus.

Discussion

The key finding of the present study is that PV-1 can suppress both apoptosis and necrosis of the infected cells. In the study, with the use of an effective test system to detect virus antiapoptotic function, we confirm an antiapoptotic function at the late stage of the virus infection cycle (simultaneously with the formation of intracellular progeny virus) in PV-1-infected cells under permissive conditions and we also found that PV-1 can grow significantly in the apoptotic cells.

Antiapoptotic function of PV

Previously, Tolskaya et al. (1995) reported that PV induces apoptosis under conditions non-permissive for virus growth although virus does not induce apoptosis under permissive conditions. Based on the observation that PV infection inhibits apoptosis of HeLa cells induced by cycloheximide or actino-
mycin D, they concluded that PV carries an apoptosis-preventing function. On the other hand, even under permissive conditions, PV infection has been found to induce apoptosis in a variety of cells (Ammendolia et al., 1999; Girard et al., 1999; Lopez-Guerrero et al., 2000). This apoptosis-inducing ability of the virus was confirmed by the finding that the expression of the PV protease genes induces apoptosis of the transfected cells in vitro (Barco et al., 2000; Goldstaub et al., 2000). Considering that (1) the use of these inhibitors to induce apoptosis does not allow normal virus replication and takes a relatively long time to induce apoptosis and (2) the experiments by Tolskaya et al. (1995) are based mostly on morphological observations, which makes it difficult to discuss the results quantitatively with the kinetics of virus multiplication, we characterized the ability of PV to prevent cell death in relation to virus multiplication to resolve the apparent discrepancy.

PV-1 grows rapidly in HEp-2 cells (Fig. 1) and does not induce apoptosis in the infected cells (Figs 2 and 3), confirming the observation of Tolskaya et al. (1995). Previously we demonstrated that the temporal incubation of HEp-2 cells in the medium containing 1 M sorbitol induces rapid and massive apoptosis of the treated cells, as judged by the classical definition of apoptosis (Kerr & Harmon, 1991) (i.e., morphology of the cells and cell nuclei as well as oligonucleosomal fragmentation of chromosomal DNA), although biochemical characterizations of intracellular signalling pathway in the treated cells are required to understand precisely the nature of sorbitol-induced apoptosis. By examining the effect of PV-1 infection on sorbitol-induced apoptosis, antiapoptotic activity of the virus was conclusively demonstrated. Comparison of the kinetics of virus multiplication (Fig. 1) and of the expression of antiapoptotic activity (Fig. 3) reveals that the expression of antiapoptotic activity is observed at the late stage of the virus multiplication cycle, simultaneously with the formation of progeny viruses. Agol et al. (2000) obtained a similar conclusion by the morphological studies on the effect of cycloheximide on the viral apoptosis-preventing function, at a drug concentration not inducing apoptosis in the infected HeLa cells but preventing the expression of virus genes. They used the Mahoney strain of PV-1 in their studies while we used the Sabin strain, indicating that the antiapoptotic function of PV-1 is not strain-specific. The apparent discrepancy between the presence of viral antiapoptotic activity and the induction of apoptosis in the infected cells of certain cell lines can be explained possibly by cell type-dependency of the viral antiapoptotic function; the function might be active in HeLa or HEp-2 cells, but not active in other types of cells reported (Ammendolia et al., 1999; Girard et al., 1999; Lopez-Guerrero et al., 2000). As to the existence of a specific antiapoptosis gene, it should be mentioned that we cannot exclude the possibility, although it is not very likely, that the observed suppression of apoptosis in the PV-infected cells might be the result of a bystander effect of the infection or the result of a constellation of multiple cellular and viral activities in the infected cells, because the antiapoptotic gene of the virus has not yet been identified.

In addition, it is noteworthy that the infection of PV in the presence of cycloheximide did not induce apoptosis in HEp-2 cells (data not shown), although HSV-1 and HSV-2 induce apoptosis under the same conditions (Koyama & Adachi, 1997; Koyama et al., 1998b). This result suggests that virion components of PV do not have any ability to induce apoptosis in the infected cells, while those of HSV-1 and HSV-2 can trigger an apoptotic response in the cells.

Characterization of sodium chloride-induced cell death and the effect of PV infection

Eukaryotic cell death has been classified into two categories, apoptosis and necrosis. Apoptosis is considered to be an active physiological process in which cells die in a tightly controlled manner under a cellular death programme, while necrosis is a passive degenerating process in which cells are killed accidentally by a toxic environment. Although death by sodium chloride is not definitely passive degeneration of the cells, the sodium chloride-treated cells can be considered to be killed by a necrosis-like mechanism, because cell death by sodium chloride is not apoptosis by the classical criteria of apoptosis. Morphology of both the dead cells (Fig. 4) and the cell nuclei (Fig. 5) are quite different from those of apoptotic cells and oligonucleosomal fragmentation (laddering) of chromosomal DNA was not observed (Fig. 2). Although the characteristics of the cells killed by necrosis are not yet as clear as those of apoptotic cells, electron microscopic observations (Fig. 6c) revealed that death by this agent shows the characteristics of liquefactive necrosis (generalized swelling, disruption of the cell membrane and condensation of chromatin without fragmentation of the nucleus; Goudie, 1985), indicating that the temporal treatment of HEp-2 cells with sodium chloride can induce one type of necrosis in HEp-2 cells. A biochemical study is now in progress to reveal a definite mechanism of sodium chloride-induced cell death.

Interestingly, PV infection also suppressed cell death induced by sodium chloride (Figs 2, 3, 4 and 5). We conclude that the virus can prevent both apoptotic and necrotic death of the infected cells. It should be noted that, although the virus has the ability to prevent death of the infected cells, these cells died at the end of infection (after the completion of progeny virus formation), probably by a non-specific loss of the regulation of the infected cell metabolism.

Multiplication of PV-1 in the apoptotic cells

In addition to the observations discussed above, PV-1 can grow in the apoptotic cells. When sorbitol-treated HEp-2 cells were infected with PV-1, the virus grew significantly, although massive fragmentation of chromosomal DNA was observed in these infected cells (data not shown). The virus yield decreased
to one-quarter of that in the untreated normal cells, but the formation of progeny virus started and reached a plateau with similar kinetics to those in the untreated cells. This result confirms our previous conclusion that virus-induced apoptosis by animal virus infection does not bring about the abortion of virus multiplication by premature death of the infected cells, but renders the infected cells to be phagocytosed by macrophages by presenting recognition signal molecules on the infected cell surface (Koyama et al., 2000b).

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