Role of virus-induced apoptosis in a host defense mechanism against virus infection

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Abstract : Many animal viruses are known to induce apoptosis in infected cells. This virus-induced apoptosis has been often described as a mechanism of host defense against virus infection, based on the finding that mutants of an insect virus with the ability to induce extensive apoptosis in some cells cannot grow in the same cells. In animal virus infection, we have shown that (1) viruses can somehow overcome this defense mechanism and that (2) virus multiplication in the apoptotic cells is not as completely suppressed as in the insect virus infection. These results suggest that, in the case of animal viruses, the virus-induced apoptosis does not play the same role in the host defense system as in insect cells. However, by examining the virus infection under the conditions comparable to the infection *in vivo*, we demonstrated the defensive role of apoptosis in animal virus infection. J. Med. Invest. 45 : 37-45, 1998

Key words : *apoptosis, antiapoptosis, virus infection, host defense mechanism, TNF, cytokine, VSV, HSV-1, HIV*

INTRODUCTION

Death of eukaryotic cells has been classified into two types ; i.e., necrosis and apoptosis (1-3). Necrosis is a passive degeneration of cells and can be defined as an accidental cell death ; cells are killed by a catastrophic toxic environment as a pathological reaction. Cell death of this type takes place through loss of integrity of the cell membrane and of intracellular organella structures without any control of cellular functions. In contrast to necrosis, there is another type of the eukaryotic cell death which is strictly regulated by a cellular genetic program (death program). In this type of cell death, cells are not killed passively, but kill themselves actively (suicide) as a physiological process. Although molecular mechanisms of this death are not yet fully understood, cells recognize certain physiological stimuli as a death signal and activate an irreversible death process resulting in a programmed cell death. Programmed cell death is a functional definition of cell death in contradistinction to necrosis, accidental cell death.

In 1972, Kerr *et al*. (4) reported a characteristic morphology of the cells dying through programmed cell death and named the cell death of this type as apoptosis. The morphology of dying cells in apoptosis is different from that in the necrotic pathway. Thereafter, Wyllie *et al*. (5) reported that apoptosis is accompanied by a fragmentation of chromosomal DNA into oligonucleosome-sized DNA. The classical definition of apoptosis is cell death in which the dying cells exhibit (1) certain morphological characteristics (shrinkage of cell volume, perinuclear condensation of chromatin and fragmentation of nuclei) and (2) biochemical characteristics, i.e., fragmentation of chromosomal DNA. However, it is noteworthy that this definition of apoptosis comes originally from the morphological aspect of cell death, being independent to any particular biological significance or functional meanings of cell death.

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INDUCTION OF APOPTOSIS BY VIRUS INFECTION

Acute virus infection usually results in the death of the infected cells. This cell death has been considered to be necrosis ; cells are killed passively by the abnormal synthesis of viral macromolecules. However, recently, some viruses have been found to induce apoptosis of infected cells. The discovery of animal virus-induced apoptosis came from the studies on E1B-19K mutant of adenovirus which is defective for virus multiplication (6). Because of its strong cytopathic effect, this adenovirus mutant was designated as a *cyt* mutant. Later, White *et al*. (7) found that this adenovirus mutant induces extensive apoptosis in infected cells and showed the presence of antiapoptosis gene in adenovirus. Chou and Roizman (8) independently found that γ 34.5 mutant of herpes simplex virus type 1 (HSV-1) induces programmed cell death in neuroblastoma cell line concomitantly with a loss of virus multiplication. Thereafter a variety of animal viruses, both DNA viruses and RNA viruses, have been found to induce apoptosis in infected cells (6-27).

Although we do not know anything about which component or process in the virus infection works as a trigger for the induction of apoptosis, there are two different kinds of apoptosis which is induced during virus infection *in vivo* (Table1). Apoptosis of the first type is induced exclusively in the virus-infected cells, not in uninfected cells. In contrast, apoptosis of the second type is considered to be mediated by a certain viral protein(s) which is excreted from the infected cells. When this protein binds to the neighboring cells via specific cell surface receptor molecules, apoptosis is induced in these cells regardless of whether they were infected or not before. As a result, massive loss of receptor-positive cells is induced in the infected individual, like a depletion of CD4-positive T cells by HIV in AIDS patients (22, 28).

In this review, we focus on the first type of apoptosis. Apoptosis of this type is induced by many viruses, both DNA viruses and RNA viruses. Interestingly, most of the RNA viruses induce apoptosis under conditions permissive for virus multiplication while DNA viruses do not. Most large DNA viruses do not induce apoptosis unless they lose the function of a certain gene by mutations in their genome (26, 27).

The biological significance of apoptosis is summarized in Table 2. As discussed below, apoptosis induced in the infected cells is expected to play a role in the host defense mechanism ; when apoptosis is induced after the virus infection, multiplication of the virus would be prematurely interrupted by the death of the infected cells (premature lysis of the infected cells). As a result, even the infected cells are killed by virus-induced apoptosis, the infected individuals are protected from a spread of progeny viruses. In this regard, virus-induced apoptosis is advantageous for organisms. However, like the infection with HIV, virus-induced apoptosis is often a trigger for the onset of disease (28). This deleterious effect is not always linked to apoptosis of the second type in Table1. For example, although HSV-1 induces apoptosis exclusively in the infected cells,

Table 1. Two kinds of virus-induced apoptosis

Triggers	Cells undergoing apoptosis	Examples
Virus infection (virion component or multiplication process)	Infected cells	Many viruses (HSV-1, VSV, etc.)
Virus gene products	Both infected and uninfected cells	HIV

HSV-1, herpes simplex virus type 1 ; VSV, vesicular stomatitis virus ; HIV, human immunodeficiency virus

Table 2. Biological significance of virus-induced apoptosis.

a) To protect the organism (1) by premature death of the virus-infected cells before the formation of progeny virus and (2) by allowing the infected cells to be removed by macrophage.

b) To damage a tissue leading to inflammation or symptoms.

we have shown that this virus can cause a fulminant hepatitis by inducing massive apoptosis in hepatocytes in infected mice (29). In this case, virus-induced apoptosis does not play a protective role but has a deleterious effect on the infected organ. In addition, this result also showed that virus-induced apoptosis is not limited to the phenomenon in the *in vitro* tissue-cultured cells, but may play some kind of role in the *in vivo* pathogenesis.

DOES APOPTOSIS WORKS AS AN ANTIVIRAL MECHANISM ?

The role of the virus-induced apoptosis in the host defense mechanism against viruses was first proposed by the studies with mutant insect viruses (30). *Autographa californica nuclear polyhedrosis virus* (AcMNPV) grew well in an SF21 cell line (derived from fall armyworm *spodoptera frugiperda*). However, p35-deficient mutants of AcMNPV could not grow in the cell line with concomitant induction of massive apoptosis. These mutant viruses could replicate normally in the TN-368 cell line (derived from cabbage looper *Trichoplusia ni*) in which the viruses did not induce apoptosis, suggesting an antiapoptotic activity of the *p35* gene as well as a close relationship between virus-induced apoptosis and abortion of virus multiplication in the infected cells. From these results, virus-induced apoptosis has been considered to lead the infected cells to premature death before completion of progeny virus formation resulting in a loss or strong suppression of virus multiplication. Furthermore, virulence toward the organism was also strongly correlated with the ability of the virus strain to induce apoptosis. When the virus was inoculated in larvae by injection, LD50 values of *p35* mutants of AcMNPV were approximately 1000-fold higher than that of wild type virus, whereas the *p35* mutants and wild type virus had similar LD50 values in *T. ni* larvae (30). Based on these results, Clem and Miller proposed that the virus-induced apoptosis can play an important role in host defense mechanism in insects (30, 31).

These authors claimed that, because insects do not have a sophisticated humoral immune system, insect cells have developed an apoptotic response to the virus infection as a host defense mechanism. This hypothetical antiviral role of apoptosis has become widely accepted not only for insect viruses but for animal viruses, since it was found that animal viruses such as adenovirus (6, 7) and herpesvirus (8) have an antiapoptosis gene and that mutant viruses without an intact antiapoptosis gene cannot grow well in host cells.

STRATEGIES OF ANIMAL VIRUSES TO OVERCOME HOST APOPTOTIC RESPONSE.

To overcome the premature death, animal viruses apparently have developed three strategies to allow their multiplication in infected cells (Table 3).

(a) Rapid multiplication.

The first one is to grow rapidly and complete the progeny formation before the onset of apoptosis. In this case, the multiplication of viruses occurs normally under the conditions where apoptosis is induced. For example, vesicular stomatitis virus (VSV) induces apoptosis of the infected cells without any observable impairment to the progeny virus production. Fig.1 shows a one-step growth curve of VSV in HeLa cells. The progeny virus appeared at 5 hours postinfection (h p.i.) and reached a plateau at 8 h p.i. Along with the multiplication of the virus, a fragmentation of the chromosomal DNA into nucleosomal oligomers was observed in the infected cells ; the apoptotic ladder could be detected at 5 h p.i. and became more obvious with time. In agreement with this frag-

Table 3 . Viral strategies to overcome premature death by apoptosis

Strategies	Examples
a) Rapid multiplication to complete progeny virus formation before the onset of apoptosis	Most RNA viruses
b) Antiapoptosis gene to suppress the onset of apoptosis	Large DNA viruses and HIV
c) Cryptic infection; Not to active a signaling pathway necessary for induction of apoptotic response in the infected cells	Persistently infected viruses

Virus genes which regulate apoptosis (either proapoptotic or antiapoptotic) are virus-specific as well as cell type-specific.

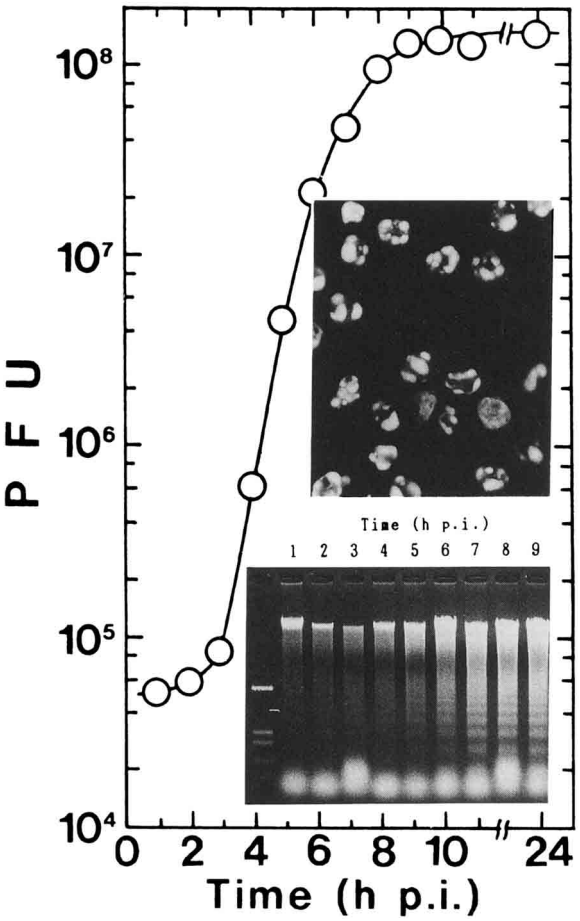

Fig.1. Virus growth, morphology of the infected cell nuclei, and oligonucleosomal DNA fragments in the VSV-infected HeLa cells. HeLa cells were infected with VSV and incubated at 37℃. At the indicated intervals, the number of progeny virus in the culture medium was determined ; separately the infected cells were harvested and the fragmented DNA was extracted from the cells by the method of Hirt (43) and in a 1.5% agarose gel. Morphology of the infected cell nuclei was examined at 8 h p.i. by the method of McGarrity (44). PFU stands for plaque forming unit.

mentation of chromosomal DNA, the characteristic morphology of apoptotic cell nuclei was observed in the infected cells.

To quantitate the chromosomal DNA extracted in the fragmented DNA fraction, cellular DNA was labeled with [^{3}H]thymidine prior to the infection and the radioactivities in fractions of total and extracted DNA were determined. Characterization of the kinetics of the induction of apoptosis revealed that apoptosis was induced with similar kinetics to that of progeny virus formation in the VSV-infected cells. This result indicates that, although cells induce apoptosis upon infection with VSV, the virus is able to overcome "premature lysis by apoptosis" of the infected cells by a rapid multiplication (18). In other words, at least in the *in vitro* tissue culture system, the induction of apoptosis does not apparently affect virus multiplication as well as virus spread to the neighboring cells.

Not only VSV, but most RNA animal viruses apparently induce apoptosis without affecting the production of progeny viruses (12, 14, 16, 21). In addition to the VSV-infected cells, we compared the kinetics of progeny virus production with that of the induction of apoptosis in the cells infected with influenza virus and obtained similar results with that of VSV (unpublished results). From these observations, we concluded that RNA viruses in general can overcome the apoptosis-mediated suppression of virus multiplication by a rapid multiplication of virus (18).

Several facts about RNA viruses support this proposal. Considering that RNA viruses have a relatively smaller number of genes than DNA viruses, it is obviously difficult to obtain an additional gene for the suppression of apoptosis ; from an evolutionary perspective, it must be easier for RNA viruses to improve the speed of virus multiplication than to acquire a new gene for antiapoptosis. Eventually, RNA viruses obtain the ability to grow quickly after infection and produce enough progeny before the onset of cell lysis by apoptosis in the infected cells. Another fact to be noted is that RNA viruses grow in the cytoplasm of the infected cells relatively independent of the functions of host cell nuclei ; this allows these viruses, not DNA viruses, to multiply rapidly.

(b) Antiapoptosis gene.

The second strategy overcoming apoptosis is to have an antiapoptosis gene. In contrast to the infection with RNA viruses, the cells infected with large DNA viruses, such as poxviruses, herpesviruses and adenoviruses, do not induce apoptosis. This lack of apoptosis is considered to be the result of a virus antiapoptosis gene which suppresses apoptotic response in the infected cells. As described above, the expression of the antiapoptosis gene, following the infection, protects the infected cells from the premature lysis by apoptosis. But, mutant viruses which lack the function of the virus antiapoptosis gene induce apoptosis upon infection, leading to the abortion of virus multiplication (31, 32).

Considering the possible importance of understanding the role of apoptosis in the life cycle of viruses, we have developed a system to identify the antiapoptosis gene. Based on our finding that sorbitol can induce apoptosis in HEp-2 cells, we used the sorbitol-treated cells to detect viral antiapoptosis

activity (33). When HSV-1-infected HEp-2 cells were treated with sorbitol, induction of apoptosis was not observed, indicating the presence of the antiapoptosis gene in HSV-1. Similar results were reported by Roizman and his colleagues (34) and, later, this gene was identified as Us 3 protein kinase gene (35). In addition, if the virus infection was carried out in the presence of cycloheximide (to avoid the synthesis of the virus antiapoptosis protein), HSV-1 induced a massive apoptosis in the infected cells (36). This result also indicates that HSV-1 can induce apoptosis without *de novo* synthesis of viral and cellular proteins after infection.

Surprisingly, human immunodeficiency virus (HIV), an RNA virus of small genome size, was discovered to have an antiapoptosis gene (37). When *vpr* gene was expressed in HEp-2 cells, sorbitol-induced apoptosis was suppressed. Considering that HIV, a member of lentiviruses, has a long incubation period (for reverse transcription, integration into host chromosome and expression of virus genome with fine regulations), it seems easy and reasonable to have an antiapoptosis gene instead of rapid multiplication of virus.

(c) Cryptic infection.

The third strategy in Table 3 is still hypothetical. Although the molecular mechanism of virus-induced apoptosis has not yet been elucidated, the trigger for this induction must be some component or process in the virus multiplication. In a persistent infection or the infection with very slowly growing viruses, there would be no serious damage of host cell functions so as to maintain the survival of infected cells. In the case where the virus does not have the antiapoptosis gene, it seems probable that the infection would be cryptic for the cells and would not trigger the induction of apoptosis.

VIRUS MULTIPLICATION IN APOPTOTIC CELLS.

Although people have considered apoptosis to be hazardous for virus multiplication, so far as we know, no one has examined the viral growth in apoptotic cells. By using the apoptotic cells induced by sorbitol, we examined the effect of apoptosis on animal virus multiplication (33). We used HSV-1 and VSV as a representative DNA and RNA virus, respectively.

As shown in Fig.2, the multiplication of HSV-1 in the apoptotic cells was affected significantly, but not completely : the virus yield decreased, but not markedly,

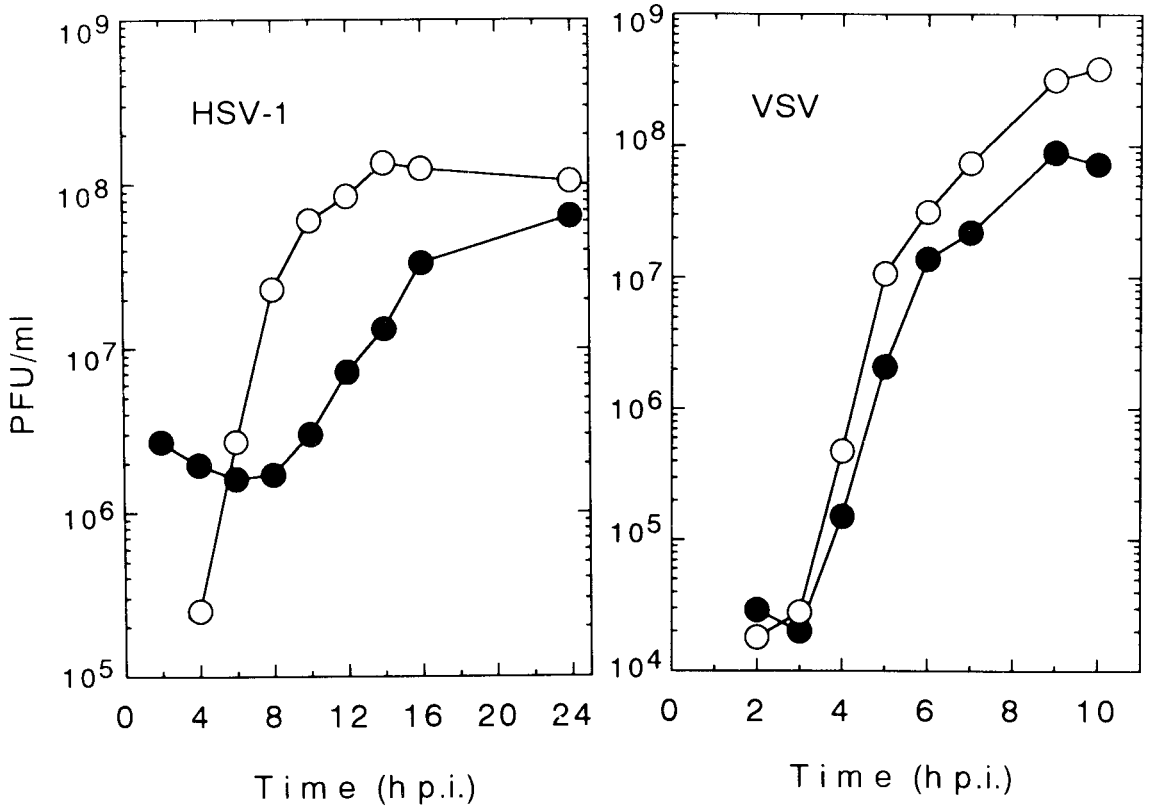

Fig.2. Virus growth in apoptotic cells. Apoptotic HEp-2 cells were prepared by incubating the cells in medium containing 1M sorbitol for 1 h at 37℃. Both apoptotic (●) and untreated normal (○) cells were infected with HSV-1 or VSV, followed by incubation at 37℃. At the indicated time, the amount of progeny virus produced in the infected culture was determined.

to below that in the control normal cells, although the rate of virus multiplication was notably affected. Similar results were obtained when VSV was used instead of HSV-1. The virus yield was decreased to approximately one tenth the amount of the control. These results indicate that the multiplication of animal viruses is affected by apoptosis of the infected cells but not as completely as that observed in most mutant insect virus-infected cells in which apoptosis is induced and virus multiplication is suppressed almost completely (31, 32).

APOPTOSIS AS A SELF-DEFENSE MECHANISM AGAINST VIRUS INFECTION

The facts that (1) most animal viruses can overcome apoptosis and (2) the multiplication of viruses cannot be suppressed completely by apoptosis might lead to the conclusion that virus-induced apoptosis does not have a significant role in a mechanism of host defense against virus infection *in vivo*. However, the facts that most viruses are able to overcome apoptosis and, especially, that most DNA viruses usually have "multiple" antiapoptosis genes suggest that the suppression of apoptosis in the infected cells would be very important for the survival of these viruses in nature.

To evaluate the role of virus-induced apoptosis in nature, we examined the virus infection in inflammatory cytokine-treated cells. At the site of virus infection *in vivo*, the infected epithelial cells produce various kinds of cytokines, such as interferons, tumor necrosis factor (TNF) and interleukines (IL-1 and IL-6), which mediate inflammatory reactions at that site (38). Among these inflammatory cytokines, IL-1 and IL-6 do not affect virus multiplication nor apoptosis in the infected cells (unpublished results). Interferons -α, -β, -γ suppressed virus multiplication markedly and, probably as a result of the inhibition of virus multiplication, interferons suppressed virus-induced apoptosis in a dose-dependent manner (manuscript in preparation).

TNF is also known to have an antiviral activity, like interferons (39-41). Fig.3 shows the kinetics of the virus multiplication and that of chromosomal DNA fragmentation (i.e., the kinetics of the induction of apoptosis) in the infected cells when the cells were pretreated with TNF (100 units TNF-α/ml) and, then, infected with VSV (42). In the untreated control cells, the progeny virus appeared at 4 h p.i. in the culture medium, and increased with time. In contrast, the multiplication of VSV was markedly suppressed in the TNF-treated cells (Fig.3A). Fig.3B shows the kinetics of the induction of apoptosis after the virus infection. The kinetics in the TNF-treated cells was quite different from that in the untreated virus-infected cells indicating that the treatment of the cells with TNF causes the cells to start apoptosis with a 1 h shorter incubation period. Because VSV overcome apoptosis simply by a rapid multiplication after infection (18), the observed acceleration of virus-induced apoptosis by TNF and the resulting earlier onset of the apoptotic response in the TNF-treated cells have a serious deleterious effect on the virus multiplication.

Essentially the same results were obtained with influenza virus-infected MDCK cells (manuscript in preparation). Previous studies by us and others have revealed that VSV and influenza virus induce

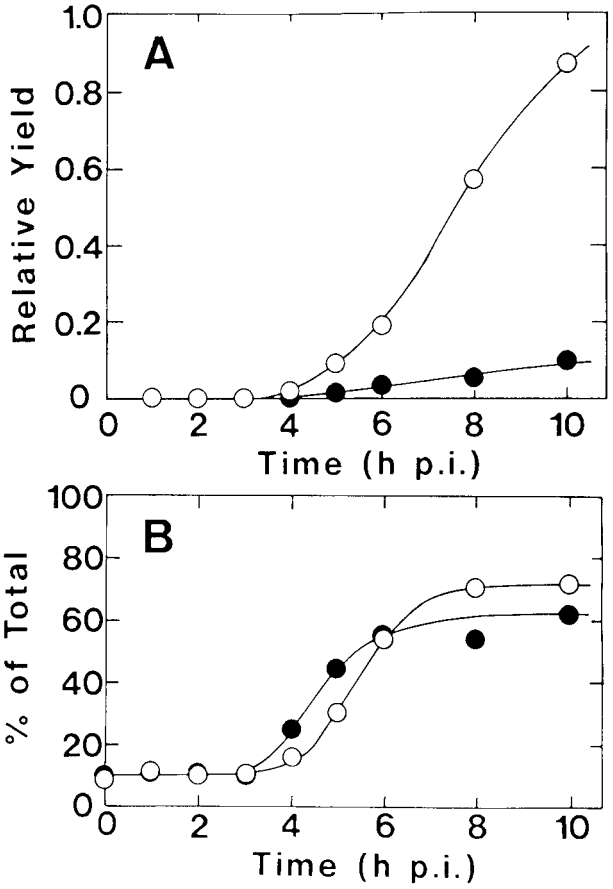

Fig.3. Effect of TNF treatment on the kinetics of the virus multiplication (A) and on the kinetics of chromosomal DNA fragmentation (B). [^{3}H]thymidine-labeled cells were treated with TNF overnight (●) or not (○) and, then, infected with VSV. At the indicated time after infection, the number of progeny viruses were determined (A) ; separately, the infected cells were harvested and the fragmented DNA was extracted. Relative amounts of fragmented DNA to total cellular DNA were determined by measuring the radioactivity in the total cellular DNA and that of the extracted DNA fragments (B).

apoptosis in cells fully permissive for virus multiplication. This indicates that the virus-induced apoptosis itself seems not to have any deleterious effect on the multiplication of these viruses *in vitro*. However, the results with TNF suggest that, *in vivo*, virus-induced apoptosis probably plays a role in a host defense mechanism against these RNA viruses in combination with TNF, one of the representative inflammatory cytokines.

CONCLUSION

The biological significance as well as the molecular mechanism of virus-induced apoptosis is still not clear. The difficulties in the characterizations probably result from a heterogeneity of virus-induced apoptosis. For each combination of virus and cell type, both the significance and mechanism of apoptosis would be different. However, as discussed in this paper, virus-induced apoptosis probably plays some role in an antiviral mechanism of the host organism ; while not solely effective, it is clearly involved in conjunction with other host defense systems in fighting against virus infection.

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