Acceleration of virus-induced apoptosis by tumor necrosis factor

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Abstract The multiplication of vesicular stomatitis virus in HeLa cells was inhibited by treating the cells with tumor necrosis factor (TNF). Comparison of the kinetics of virus multiplication and that of virus-induced apoptosis in the TNF-treated cells revealed that the antiviral effect of TNF is accompanied by a rapid induction of apoptosis in the cells upon infection, suggesting that TNF can inhibit virus multiplication by accelerating an apoptotic response in the infected cells.

Key words: Tumor necrosis factor; Apoptosis; Vesicular stomatitis virus

1. Introduction

Virus infection induces a group of cytokines which mediate an inflammatory reaction at the site of the infection. Tumor necrosis factor (TNF) is one of these inflammatory cytokines and is considered to take some roles in a host defense mechanism by interacting with a variety of immune and non-immune cells [1]. This cytokine can suppress the multiplication of viruses in the infected cells directly like the action of an interferon [2–5], although the mechanism of this antiviral activity has not yet been elucidated. No specific step in virus multiplication has been found to be inhibited by TNF.

Apoptosis or programmed cell death is one type of animal cell death in which cells die by an active cellular process under the genetic control of the cells ('death program') [6]. Although many animal viruses are known to induce an apoptotic response in infected cells, the significance of virus-induced apoptosis is not yet clear [7]. Previous studies on the effects of apoptosis on the in vitro replication and the in vivo infectivity of a baculovirus revealed that a host apoptotic response provides protection against virus infection at the organismal level [8], suggesting that the virus-induced apoptosis can have some role in a host defense mechanism [9,10]. In agreement with this hypothesis, apoptotic cell death concomitantly brings an abortion of the progeny virus production in some cases of animal virus infections in vitro [11–13], although there are a number of exceptions, especially in the infection with RNA viruses [14–17].

To understand the antiviral mechanism of TNF, we characterized the multiplication of vesicular stomatitis virus (VSV) in TNF-treated HeLa cells in relation to the kinetics of virus-induced apoptosis in host cells.

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2. Materials and methods

2.1. Cells and virus

HeLa cells were grown in minimal essential medium with Earle's salts (MEM) supplemented with 10% newborn bovine serum. VSV (New Jersey strain) was used throughout the experiments.

2.2. Cytokine treatment

Recombinant TNF-α and recombinant consensus interferon α were kindly provided by Amgen (Thousand Oaks, CA, USA). Cytokine treatment and virus infection were carried out as follows: confluent monolayers of HeLa cells were preincubated in MEM containing 0.1% bovine serum albumin (BSA) and TNF or interferon α for about 16 h. The treated cells were infected with VSV at a multiplicity of infection (MOI) of 10 or 12 by essentially the same procedure as described previously [17,18]. The infected cells were incubated in the medium containing BSA and cytokine for the indicated period.

2.3. Detection of apoptosis

The morphology of cell nuclei and the fragmentation of chromosomal DNA were examined by the methods described previously [19].

3. Results

3.1. Effect of TNF on virus multiplication in HeLa cells

Fig. 1 shows the antiviral activity of TNF-α in comparison with that of interferon α. When HeLa cells were infected with VSV, the progeny virus yields decreased with increasing concentrations of the agent in the TNF-treated cells, although not so markedly as with interferon. Although TNF can induce the synthesis of interferon in some cells [1], the observed antiviral activity of TNF is not mediated by the production of interferon, because the addition of anti-interferon serum did not affect this activity of TNF (data not shown). In addition, the TNF-treated cultures usually showed stronger cytopathic effects (CPE) by the virus infection than the untreated cultures (see below) while the CPE of interferon-treated cultures was markedly suppressed. The clear difference in the CPE between TNF- and interferon-treated cells supports that the antiviral mechanism of TNF and that of interferon are distinct.

3.2. Virus growth in TNF-treated cells

Fig. 2 shows the one-step growth curve of VSV in the TNF-treated and untreated Hela cells. In the untreated cells, the progeny virus substantially appeared at 4 h post infection (p.i.) in the culture medium, increased with time and reached a plateau after 10 h p.i. In the TNF-treated cells, the progeny virus appeared at the same time p.i. as in the untreated cells, but gradually stopped increasing; the final yield under the conditions (100 units TNF-α/ml) was approximately one tenth of that in the untreated cells. In addition, although incubation of HeLa cells with TNF-α does not induce significant morphological changes of the cells, the TNF-treated cultures showed stronger CPE upon infection with VSV than the untreated cultures, e.g. a shrinkage of the infected cells and

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plasma membrane blebbing and ballooning (data not shown). These morphological characteristics are known to be those of apoptotic cells.

3.3. Nuclear morphology of infected cells

Apoptosis has been defined morphologically by cell shrinkage and nuclear fragmentation and biochemically by fragmentation of chromosomal DNA into nucleosomal oligomers [6]. As reported previously [17], infection with VSV induces massive apoptosis in the infected cells. Fig. 3 shows the morphology of the infected cell nuclei at various times after infection. Although even uninfected HeLa cells (Fig. 3A) usually contain a small fraction of apoptotic cells, a significant amount of infected cells with unusual condensation of chromatin appeared at 3 h p.i. in the TNF-treated cells (Fig. 3B); the chromatin condensation as well as the fragmentation of infected cell nuclei became very evident at 4 h p.i. (Fig. 3C). In contrast, the apoptotic cells appeared at about 4 h p.i. in the control culture without TNF treatment (Fig. 3D). At 6 h p.i., both the TNF-treated (Fig. 3E) and untreated (Fig. 3F) cells showed large numbers of apoptotic cells in the infected culture. The treatment of HeLa cells with TNF alone (without infection) did not induce these morphological characteristics of apoptosis (data not shown).

3.4. DNA fragmentation in infected cells

In agreement with these results, the degradation of chromosomal DNA into oligonucleosome-sized fragments occurred with a shorter incubation period in the TNF-treated cells than in the untreated cells. Fig. 4A shows that the characteristic DNA ladder was clearly visible at 5 h p.i. in the TNF-treated cells, while it was visible but not so evident at 5 h p.i. in the untreated cells. To determine the detailed kinetics of this increase in the amount of chromosomal DNA extracted in the fragmented DNA fraction, cellular DNA was labeled with [^{3}H]thymidine prior to virus infection; at the indicated intervals after infection, the radioactivities in fractions of total and extracted DNA were determined separately and the relative amounts of fragmented DNA in the total DNA were calculated. As shown in Fig. 4B, the amount of fragmented DNA started increasing at approximately 4 h p.i. and reached a plateau at 8 h p.i. in the untreated cells, as reported previously [17]. In contrast, the increase started at 3 h p.i. and was completed at approximately 6 h p.i. in the TNF-treated cells, indicating that, when the TNF-treated cells were infected with VSV, the virus-induced apoptosis occurred with an incubation period 1 h shorter than in the untreated cells.

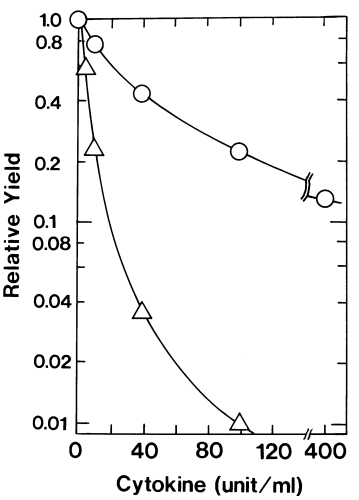

Fig. 1. Antiviral effect of TNF-α and interferon α. HeLa cells were incubated in MEM containing 0.1% BSA and various concentrations of TNF-α (○) or interferon α (△) for 14 h prior to the infection. The cells were infected with VSV at an MOI of 12 and were incubated in the medium with the same concentrations of the cytokine. Virus yields were determined by measuring the amounts of progeny virus in the culture medium at 8 h p.i.

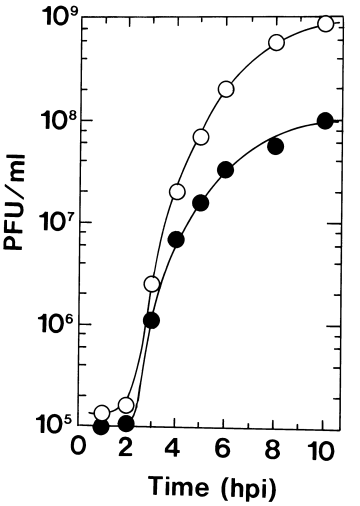

Fig. 2. One-step growth curve of VSV in the TNF-treated and untreated cells. HeLa cells were incubated with TNF-α (100 units/ml) for 16 h prior to the infection. The cells were infected with VSV at an MOI of 10 and were incubated in the presence (●) or absence (○) of TNF-α. At intervals, the amounts of the progeny virus in the medium were determined.

To examine the effect of apoptosis on the multiplication of VSV, we prepared apoptotic cells by treating the cells with 1 M sorbitol [19]. Although VSV could grow in these apoptotic cells with an almost similar eclipsed period an in the untreated normal cells, virus multiplication was obviously suppressed; the final yield of progeny virus decreased to below one fifth of the normal cells.

4. Discussion

Cells of most cell types express TNF receptors on their surface. After binding to these receptors, TNF can activate signal transduction pathways to induce pleiotropic biological effects, including apoptosis, antiviral activity, and activation of the transcription factor NF-κB [20]. As shown in Fig. 1, the multiplication of VSV is suppressed dose-dependently in TNF-treated HeLa cells, although Mestan et al. [3] described in their earlier work, without showing experimental data, that VSV can grow normally in TNF-treated HeLa cells. To elucidate the mechanism of antiviral activity of TNF, we characterized the kinetics of virus growth in relation to that of virus-induced apoptosis in the TNF-treated cells.

Previously we showed that VSV can induce massive apoptosis in infected cells and the production of progeny virus occurred with almost the same kinetics as that of DNA fragmentation, indicating that the production of progeny virus is limited after the induction of the apoptotic response in infected cells. From these results we concluded that, by a rapid multiplication after infection, VSV can escape apoptotic cell

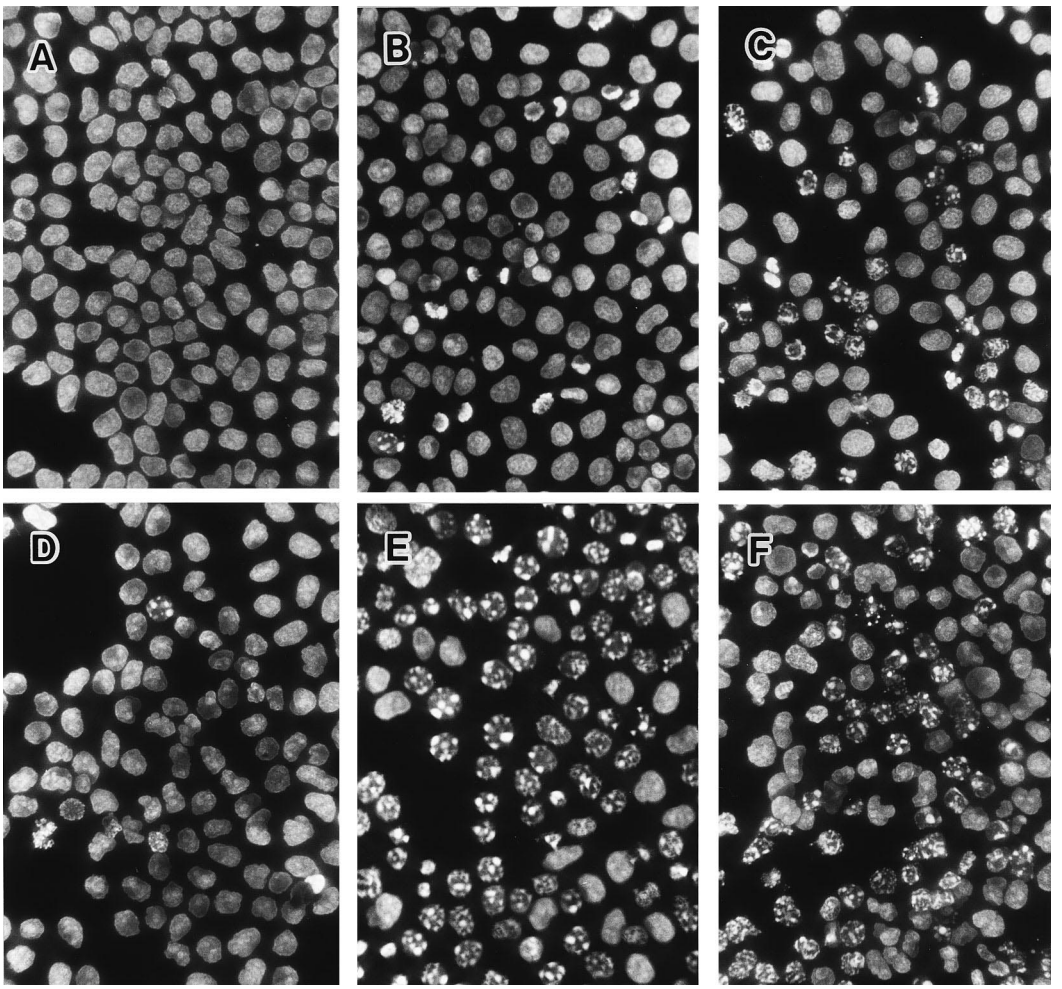

Fig. 3. Morphology of the infected cell nuclei. See legend to Fig. 2. The infected cells were fixed with methyl alcohol/acetic acid (3:1) and stained for 10 min with the DNA binding dye Hoechst 33258 (0.05 μg/ml) according to the method of McGarrity [22]. A: Uninfected control without TNF treatment. B: TNF-treated cells at 3 h p.i. C: TNF-treated cells at 4 h p.i. D: Untreated cells at 4 h p.i. E: TNF-treated cells at 6 h p.i. F: Untreated cells at 6 h p.i.

damage and the resulting suppression of virus growth in permissive host cells [17]. In the present study, we demonstrated that treatment of HeLa cells with TNF causes the cells to start apoptosis with a 1 h shorter incubation period after the onset of infection (Figs. 3 and 4). Although we do not know how critical this 1 h period is for the multiplication of VSV, the observed restriction in virus multiplication can be the result of the earlier onset of the apoptotic response in TNF-treated cells, because the multiplication of VSV is impaired in apoptotic cells.

However, the results in Figs. 3 and 4 also indicate that not all cells showed the characteristics of apoptotic cells (i.e. morphology of cell nuclei and fragmentation of chromosomal DNA). This heterogeneity in the apoptotic response of infected cells is probably due to heterogeneity in the stages of virus multiplication in each cell in these infected cell populations. In addition, it should be noted that the observed heterogeneity cannot exclude that apoptosis was induced in all cells in these cultures, because quantitation of cells dying through the apoptotic pathway is difficult; the characteristic marker currently available to detect apoptosis is almost the final outcome of biological processes toward programmed cell death. Further characterization of the apoptotic pathway is necessary to detect a key step in a specific aspect (e.g. antiviral effect) of apoptosis; at least, it is obvious that the multiplication of VSV is not inhibited directly by the nuclear fragmentation or degradation of chromosomal DNA, because VSV can grow even in enucleated cells [21].

Although we cannot exclude the possibility that the observed acceleration of apoptosis does not affect virus multiplication and is independent of the observed restriction of virus multiplication by TNF, this possibility is less likely because (1) the multiplication of VSV is restricted in apoptotic cells and (2) essentially the same results were obtained with influenza virus-infected MDCK cells; influenza virus A/Aichi induces apoptosis with a shorter incubation period in TNF-treated cells than in untreated cells with a concomitant reduction of the progeny virus yield (Koyama, unpublished results). These results are most consistent with the idea that the acceleration of apoptosis accounts for the observed restriction of virus multiplication in the TNF-treated cells.

Previous studies by us and others revealed that VSV and influenza virus induce an apoptotic response in cells fully permissive for virus multiplication, suggesting that the virus-induced apoptosis itself seems not to have any deleterious effect on the multiplication of these viruses in vitro [14,16,17]. How-

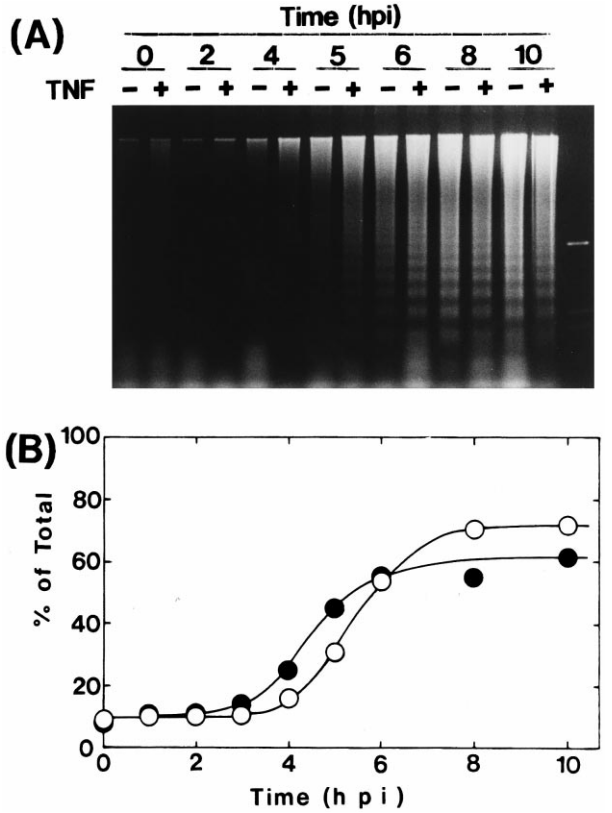

Fig. 4. DNA fragmentation in VSV-infected cells. [^{3}H]Thymidine-labeled HeLa cells were treated with TNF-α and infected with VSV as described in the legend to Fig. 2. At the indicated intervals, the cells were harvested and the fragmented DNA was extracted, precipitated and analyzed in a 1.5% agarose gel (A). For the quantitative analysis, the radioactivities in fractions of total and fragmented DNA of the TNF-treated (●) or untreated (○) cells were determined separately as described previously [19].

ever, the present study suggests that, in vivo, virus-induced apoptosis probably has a certain role in a host defense mechanism against these RNA viruses in combination with TNF, one of the representative inflammatory cytokines.

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