The physiological role of the induction of apoptosis in influenza virus-infected cells

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学 位 論 文 要 旨

ABSTRACT

HeLa cells undergo typical apoptosis upon influenza A virus infection. However, the mechanism and physiological significance of this phenomenon have remained to be solved. In the present study, I assessed these two issues and obtained evidence as follows.

In influenza virus-infected cells, the amount of cell surface Fas, the specific receptor for the apoptosis inducer Fas-ligand, increases at the transcription level prior to their death. I showed that both Fas-ligand and Fas were simultaneously induced upon virus infection, and that apoptosis was significantly inhibited by an antagonistic anti-Fas-ligand antibody. These results suggest that influenza virus-infected cells, which possess both Fas and Fas-ligand on the cell surface, bind to and induce apoptosis each other.

The amount of virus released into culture medium does not change even when apoptosis of virus-infected cells is inhibited. In general, apoptotic cells are rapidly eliminated by phagocytosis. I therefore co-cultured influenza virus-infected cells with macrophages, and examined the extents of phagocytosis and virus growth. Virus-infected cells at early infectious stages were efficiently phagocytosed by macrophages depending on the occurrence of apoptosis, and virus release completely ceased in the presence of macrophages. Furthermore, this inhibition required direct contact between the two cell types, and influenza

virus particles together with host cells were present in phagosome-like structures within macrophages as examined by electron microscopy. These results suggest that influenza virus, together with host cells, is engulfed by macrophages and inactivated. I propose that the induction of apoptosis in influenza virus-infected cells occurs as a part of the self-defending mechanism for protecting the organism from viral invasion.

Introduction

HeLa cells undergo typical apoptosis upon influenza A virus infection. The amount of Fas increases at the transcription level in influenza virus-infected HeLa cells prior to apoptosis induction. Moreover, the addition of an apoptosis-antagonizing anti-Fas monoclonal antibody partially inhibits apoptosis, suggesting the involvement of Fas in apoptosis induction. Fas is needed to be ligated with Fas-ligand for transmitting apoptosis signals, but HeLa cells normally do not produce Fas-ligand. It is thus important to determine a change in the amount of Fas-ligand in influenza virus-infected HeLa cells in order to clarify whether or not the Fas-ligand/Fas system is involved in apoptosis induction.

Although it has been postulated that the death of virus-infected cells should lead to spread of virus progeny, inhibition of apoptosis in influenza virus-infected cells by a caspase inhibitor does not affect the amount of replicated virus released into the culture medium. Therefore , the physiological role of apoptosis induction in host cells upon influenza virus infection has remained unclear. Since cells undergoing apoptosis are engulfed by phagocytic cells, clarifying the consequences of phagocytosis of apoptotic virus-infected cells may be required for understanding the physiological role of the induction of apoptosis. It has been recently reported that phagocytosis of influenza virus-infected cells by dendritic leads to antigen presentation towards CD8-positive cytotoxic T lymphocytes. On the other hand, phagocytosis of those cells by macrophages did not result in stimulation of T lymphocytes. From such a point of view, I tried to determine the consequence of phagocytosis of influenza virus-infected cells by macrophages in terms of the efficiency of virus growth. In the present study, I aimed at elucidating the mechanism and physiological significance of apoptosis in influenza virus-infected cells.

Results

Mechanism for the induction of apoptosis in influenza virus-infected cells

It was previously showed that expression of cell surface Fas increases at about 6 h after infection, but changes in Fas-ligand expression have not been determined. Therefore, I first examined the presence of Fas-ligand on the surface of influenza virus-infected HeLa cells by flow cytometry. The amount of cell surface Fas-ligand also increased with a time course similar to that for Fas. To examine whether Fas and Fas-ligand are co-expressed on the surface of virus-infected cells, the cells were simultaneously treated with the two primary antibodies and analyzed. The results showed that about 70% of the cells were positive for both Fas and Fas-ligand at 24 h after infection, indicating that Fas and Fas-ligand are simultaneously induced and appear on the surface of virus-infected cells. Furthermore, the addition of an antagonistic anti-Fas-ligand antibody significantly inhibited apoptosis of virus-infected cells. These results indicated that apoptosis of influenza virus-infected cells is mediated by the Fas-ligand/Fas system. It is likely that apoptosis is induced when the virus-infected cells attach to each other bringing about ligation of Fas-ligand to its receptor Fas.

Phagocytosis of influenza virus-infected cells by macrophages

Since cells undergoing apoptosis are eventually eliminated by phagocytosis, it might be the case with influenza virus-infected cells. I anticipated that phagocytosis of virus-infected cells, if it happens, leads to the inhibition of virus growth. I thus co-cultured virus-infected cells with macrophages and examined the extent of phagocytosis. The results showed that virus-infected cells at early stages of infection were efficiently phagocytosed by macrophages. I next examined whether this phagocytosis requires the occurrence of apoptosis in virus-infected cells. To test this, z-VAD-fmk, a caspase inhibitor, was added to the culture medium from the beginning of infection. The drug reduced the extent of apoptosis, and at the same time, phagocytosis of z-VAD-fmk-treated cells decreased to a similar extent. This indicated that macrophages phagocytosed virus-infected cells that were undergoing apoptosis.

Inhibition of virus growth in the presence of macrophages

I next examined whether virus growth is affected by a co-culture with macrophages. HeLa cells at 9 h of infection were transferred to a macrophage culture. The co-culture was further maintained, and the virus titer in the culture medium was determined. The virus titer in the medium of a culture without macrophages increased as culture time was prolonged, whereas the virus titer did not increase when the cells were maintained with macrophages. These results indicated that the growth of influenza virus was completely inhibited in the presence of macrophages. To next examine whether this macrophage effect requires direct interaction of the two cell types, virus-infected cells and macrophages were placed on opposite sides of a permeable membrane , and the virus titer was determined. The virus growth was not inhibited at all under this condition. This result showed that macrophages needed direct contact with virus-infected cells for inhibiting virus growth. When a mixed culture of virus-infected cells and macrophages were examined by electron microscopy, phagosome-like structures were observed in macrophages, and the presumed phagosomes were often associated with influenza virus particles. These results indicated that influenza virus was engulfed by macrophages together with its host cells. It is likely that inhibition of virus growth is due to phagocytosis.

Discussion & Conclusion

I first determined changes in the amount of Fas-ligand in influenza virus-infected cells in order to clarify whether the Fas-ligand/Fas system is involved in apoptosis induction. Fas-ligand and Fas were simultaneously induced and appeared on the cell surface upon virus infection. Furthermore , the addition of an antagonistic anti-Fas-ligand antibody reduced the extent of apoptosis. From these results , it is concluded that apoptosis of influenza virus-infected cells is triggered by the Fas-ligand/Fas system. I propose a model for apoptosis induction as shown in Fig.1.

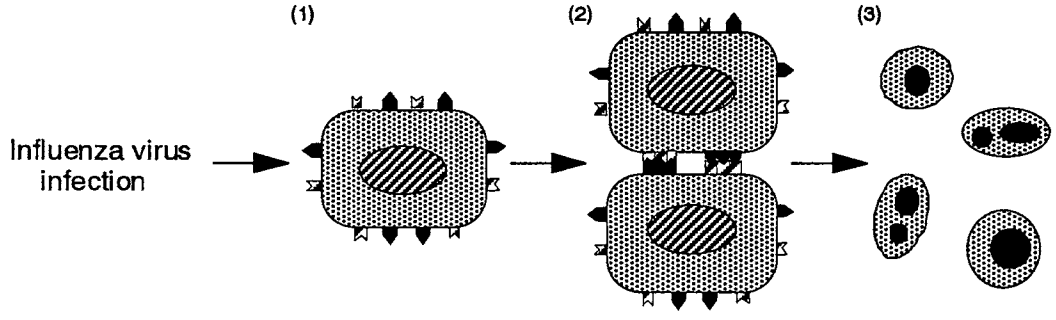

Fig. 1 A model for apoptosis induction in influenza virus-infected cells.

(1) The amount of cell surface Fas (▨) and Fas-ligand (▲) increases upon virus infection. (2) The cells containing these proteins attach to each other bringing about binding of Fas-ligand to its receptor. (3) Such cells undergo apoptosis.

Fas-ligand is undetectable in normal HeLa cells, but Fas pre-exists in those cells. However, uninfected HeLa cells do not undergo apoptosis in the presence of an agonistic antibody. Therefore, activation of the production of both Fas-ligand and Fas is prerequisite to the induction of apoptosis in influenza virus-infected cells.

In previous study, the production of virus progeny was not affected when apoptosis was inhibited in influenza virus-infected cells. In general, since apoptotic cells are rapidly eliminated by phagocytosis, I examined the possibility that influenza virus-infected cells are engulfed and, if so, virus growth is altered. The results showed that virus-infected cells were efficiently engulfed by macrophages, depending on the occurrence of apoptosis, and this resulted in complete inhibition of virus growth. Electron microscopic analyses suggested that influenza virus was incorporated into macrophages and inactivated. From these results, I presume that influenza virus-infected cells undergoing apoptosis are engulfed by macrophages at early stages of infection, and that this leads to significant inhibition of virus growth. It can be considered that apoptosis induction in influenza virus-infected cells is a part of the self-defense mechanism for protecting the organism from viral invasion (Fig. 2).

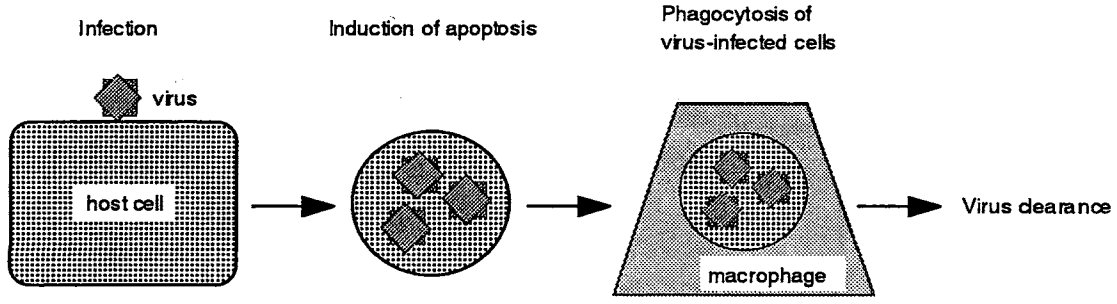

Fig. 2 Virus clearance by apoptosis-dependent phagocytosis of influenza virus-infected cells.

学位論文審査結果の要旨

冨士本一平氏から提出された学位論文について，上記５名の審査委員による査続の後に平成12年2月1日に口頭発表会が行われた。同日に最終の審査委員会が開かれ，以下の理由により当該論文は博士(薬学)の学位に値すると判定された。

本論文は，インフルエンザウイルス感染で誘導されるアポトーシスの機構と意義に関する研究結果を記述したものである。多くのウイルスが宿主細胞にアポトーシスを誘導することが知られるが，その仕組みが解明された例は少ない。また，ウイルス感染細胞がアポトーシスを起こすことの意味は不明であった。

冨士本氏は，インフルエンザウイルス感染細胞ではアポトーシス誘導因子Fasリガンドとその受容体Fasの生産がともに高まり，両者を持つようになった細胞が接触して互いにアポトーシスを誘導し合うという機構を示した。他のウイルスを含め，これまでアポトーシス誘導機構が明快に示された例はなく，新しい成果と評価される。さらに，ウイルス感染細胞がアポトーシス依存的にマクロファージに貪食されることにより，ウイルス増殖が停止することが明らかにされた。アポトーシス細胞の貪食は，周辺組織に害を与えないように死細胞を除去するための現象だと理解されてきた。冨士本氏の発見により，同現象が病原体を排除するための積極的な生体防御機構である可能性が生まれた。

本研究は培養細胞を用いて行われたものであり，動物実験による検証が必要である。しかし，これらの成果はウイルス病に関する基礎および応用研究に新たな視点を与えるものと評価される。