Role and Pathological Significance of Apoptosis Induced by Influenza Virus Infection

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Abstract: The role and pathological significance of apoptosis in an influenza virus-infected host has been hotly debated. Influenza virus-induced apoptosis was primarily thought to be a host defense mechanism to limit virus replication and eliminate viruses from the host; however, the virus has mechanisms not only to overcome but to utilize apoptosis for its efficient replication. Virus-induced apoptosis also plays a role in developing symptoms. Understanding the mechanisms underlying virus-induced apoptosis could enable us to conquer the threat of influenza.

Keywords: Influenza, apoptosis, inflammation, cytokine, signal transduction.

I. INTRODUCTION

Apoptosis is characterized by nuclear condensation and fragmentation of cell bodies into apoptotic bodies that are rapidly engulfed by macrophages or neighboring cells [1]. Apoptosis occurs in many pathological processes, such as cancer cells, inflammatory cells, microbe-infected cells, metabolically abnormal cells and so on [2].

A variety of viruses have been shown to induce apoptosis or programmed cell death in a host [3-5]. Many strains of influenza A virus, including highly pathogenic avian influenza A virus, as well as B virus have been shown to induce apoptosis in a variety of cells both *in vitro* and *in vivo* [6-9].

It has been thought that apoptosis is primarily a host defense mechanism, limiting virus replication and eliminating viruses from the host; however, much evidence has shown that many viruses have mechanisms to overcome apoptosis and are able to replicate efficiently before apoptosis completes, and to utilize the mechanism of apoptosis [4, 10, 11].

This paper summarizes recent information on influenza virus-induced apoptosis and offers potential therapy.

II. INFLUENZA VIRUS-INDUCED APOPTOSIS IN NATURAL HOSTS AND EXPERIMENTAL ANIMALS

Apoptosis was observed in alveolar epithelial cells, spleen lymphocytes and lung leukocytes in specimens from patients who had died from infection with the highly virulent H5N1 virus [12, 13]. H5N1 virus infection caused notable apoptosis of activated dentritic cells (DCs) in the lung and draining lymph nodes in macaques [14]. Virulent avian influenza A viruses induced apoptosis in vascular endothelial cells of chickens [15].

Many influenza viruses induced apoptosis in a variety of primarily cultured cells or cell lines, such as lymphocytes, bronchiolar epithelial cells, chicken embryonic cells, macrophages, and myeloid DCs [7, 16-20].

This evidence indicates that influenza virus infection causes apoptosis in natural hosts, and apoptosis plays roles in the pathogenesis of influenza virus by destroying cells. Since apoptotic cells were less frequent than cells that harbored virus antigens, apoptosis seems to be either a direct or an indirect consequence of virus infection, [16, 21].

III. MECHANISMS OF INFLUENZA VIRUS-INDUCED APOPTOSIS

1) Host Factors

H3N2 virus infection increased the expressions of both Fas and Fas ligand (FasL) on HeLa cells, thereby Fas/FasL ligation on the surface of virus-infected cells was predicted to cause apoptosis [6, 22, 23]. Inhibitory anti-Fas or FasL antibodies partially suppressed virus-induced apoptosis [23, 24]. Apoptotic human peripheral lymphocytes induced by H1N1 or H3N2 virus expressed Fas in high density, whereas monocyte-derived macrophages (MDM) increased FasL expression after virus exposure, also implicating the role of Fas/FasL ligation [16]. Exposure of human MDM to H5N1 virus upregulated tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and tumor necrosis factor-α (TNF-α), which sensitized co-cultured T-cells to apoptosis [25]. In mice infected with H1N1 virus, TRAIL and its ligand of DR5 were induced in NK, CD4+ and CD8+ T cells [26]. Administration of inhibitory anti-TRAIL monoclonal antibody to mice significantly delayed virus clearance, implying that TRAIL plays a role in the immune response to virus infection [26].

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Influenza virus infection activated initiator caspases-8, 9 and effecter caspase-3 [27, 28]. H5N1 virus also significantly activated caspases-3, 8, and 9 in primary alveolar epithelial cells [20]. Transfection of H5N1-encoded nonstructural protein 1 (NS1) into epithelial cells also activated caspases-3, 7, and 8, as well as FasL expression [29].

The dominant negative form of double-stranded RNA-activated protein kinase (PKR) in HeLa cells suppressed H3N2 virus-induced cell death and Fas expression [30]. Activated PKR itself induced several pro-apoptotic genes, including p53, Bax, Fas, and recruitment of caspases-8, 9 by the cytoplasmic protein Fas-associated death domain (FADD) [31-33]. Interferon (IFN)-α/β sensitized the fibroblasts to FADD-dependent apoptosis, which is possibly regulated by PKR [34]. H3N2 virus infection activated apoptosis signal-regulating kinase1 (ASK1) in human bronchial epithelial cells, which resulted in the phosphorylation of c-Jun-NH_2-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) [35]. $ASK1^{-/-}$ mouse embryonic fibroblast (MEF) was defective in caspase-3 activation after influenza virus infection, indicating that influenza virus-induced apoptosis depends on ASK1 [35].

2) Virus Factors

Neuraminidase (NA) facilitated the cleavage of latent transforming growth factor (TGF)-β into the active form, which induces apoptosis in cells [36]. Monocyte-expressing PB1-F2, encoded by the +1 reading frame of PB1, underwent apoptosis, suggesting that this protein kills immune cells against influenza virus [37]. PB1-F2 permeabilized and destabilized the mitochondrial membrane, leading to leakage of cytochrome C [38-40]. While NS1 reportedly induced apoptosis [29, 41], mutant H1N1 virus deleted with NS1 increased apoptosis in virus-infected cells [42]. The latter is possibly through its ability to inhibit type I IFN production [43], which potentiates cells to apoptosis [24]. NS1 seems to be a universal antagonist of double-stranded RNA-induced signaling processes, including IFN [10]. Recently, NS1 reportedly activated phosphatidylinositol-3-kinase (PI3K), which resulted in the activation of its effecter of Akt, a survival signal cascade [44, 45]. This led to subsequent inhibition of caspases-3, 9 and glycogen synthase-kinase 3β and limitation of the virus-induced cell death program [44, 45].

The above evidence indicates that a variety of host and viral factors are involved in virus-induced apoptosis. Further *in vivo* experiments will be required to understand the precise mechanisms of how each pathway works either mutually or independently.

IV. ROLE OF APOPTOSIS IN INFLUENZA VIRUS REPLICATION

Alveolar macrophages and neutrophils phagocytosed influenza virus-infected cells by recognizing phosphatidylserine (PS), which is externalized during apoptosis [46, 47]. Phagocytic elimination of virus-infected cells almost completely inhibited virus growth *in vitro* [48]. When phagocytic activity was inhibited by PS-binding protein of annexin V, inflammation and lethality were enhanced [49]. Apoptosis is thus regarded as a host defense mechanism against virus infection [50].

However, influenza virus may efficiently replicate before apoptosis completes [51], or make more viruses by removing apoptotic cells that have already been used to replicate [52]. Influenza virus production was impaired by overexpression of Bcl-2 [7, 53], inhibition of caspase-3 [28], inhibition of Raf/MEK/ERK cascade [54], or inhibition of PI3K/Akt cascade [55]. The impairment of virus growth correlated with the retention of viral ribonucleoprotein complexes (RNPs) in the nucleus irrespective of treatments. Nuclear export of RNP complexes may depend on rather passive diffusion due to apoptosis than active export machinery because this process was not inhibited by a specific inhibitor of nuclear export [28]. Viral replication was also partially impaired by inhibiting nuclear factor κB (NF-κB), which resulted in suppression of the expression or function of TRAIL or Fas/FasL, the expression of which is reportedly required for efficient viral propagation [56].

The above evidence indicates that influenza virus acquires the ability to utilize apoptosis for its propagation. While the virus may be able to resist or rather utilize apoptosis for efficient propagation in the early phase of apoptosis, the host may utilize apoptosis to eliminate dying cells completely in the late phase.

V. LINKAGE OF INFLUENZA VIRUS-INDUCED APOPTOSIS TO SEVERITY OF DISEASE

Marked activation of pro-inflammatory and cell death pathways after influenza virus infection have a definitive role in the pathogenesis of influenza [57]. The chemokine regulated on activation, normal T cells expressed and secreted (RANTES) was induced in epithelial cells after H3N1 virus infection [58]. Caspase inhibitors enhanced the expression of pro-inflammatory cytokine release in epithelial cells after influenza virus infection, suggesting that apoptosis limits cytokine release [17]. H5N1-infected macrophages strongly induced apoptosis in co-cultured T-cells [25] and virus infection induced apoptosis in numerous leukocytes in the lung [13], suggesting that induction of apoptosis relates to lympho- and leukopenia, respectively.

H5N1 virus replicated in monocyte-derived and myeloid DCs and caused cell death, which could be inhibited by pretreatment of DCs with IFN-α and Toll-like receptor (TLR) ligands [19]. Virus-induced massive death of DCs may impair the induction of virus-specific immune responses. Alternatively, IFN-α induced by H5N1 virus may potentiate viral production from DCs and lead to sustained viremia by inhibiting cell death of DCs. Apoptosis of activated DCs in the lungs and draining lymph nodes was observed in macaques infected with H5N1 virus, which resulted in the deregulation of adaptive immune responses [14]. Mice infected with fully reconstructed 1918 H1N1 virus showed marked and sustained activation of pro-inflammatory and cell-death pathways, while those infected with less virulent viruses induced less host immune response accompanied by less severe pathology [59].

The above evidence suggests that cell death responses contribute to severe immunopathology.

VI. ANTI-APOPTOTIC THERAPY

JNK and PI3K cascades have been considered to be an anti-apoptotic target [10, 11]. Although JNK and PI3K cas-

cades play pro- and anti-apoptotic roles, respectively, inhibitors of these cascades resulted in strong impairment of viral propagation [10, 11]. Inhibition of NS1 to prevent activation of the PI3K cascade could also be a useful way of restricting influenza A virus replication [60]. Caspase-3 might be a target for anti-viral intervention, since compiling studies in animals infected with several viruses showed that the conditions were designed to inhibit caspases and reduce disease severity, even though there were conflicting effects on the viral titer [5]. Another novel strategy to reduce disease severity has been discussed to raise the phagocytic activity of macrophages or neutrophils that engulf virus-induced apoptotic cells [50].

These compounds targeting cellular factors meet the criteria for potential anti-influenza agents: not toxic to a variety of cell types and no tendency toward induction of resistant virus variants [10, 11]; however, there are difficulties in using compounds as they may globally affect other related factors, or their effect may be limited to specific strains.

Alternatively, anti-apoptotic therapy may support continued viral replication, worsening the clinical outcome or spreading infection. Furthermore, the overall effects by using these compounds might be difficult to anticipate, since the influence of one pathway can thereafter affect multiple signal transduction cascades through feedback loops. We therefore have to prudently take into consideration the balance between disease severity and the potential adverse effects of these compounds.

VII. CONCLUDING REMARKS

Apoptosis has been offering a novel aspect on influenza virus research. Various signaling pathways of apoptosis induced by influenza virus infection have been described in the past decade and new pathways are being unraveled owing to the remarkable advancement of general research on apoptosis. As the compiled evidence indicates a significant role of virus-induced apoptosis in developing diseases, as described above, defining its mechanisms will provide novel therapeutic aids against influenza. To confront the devastating threat of recurrent pandemics of influenza viruses, it is urgently necessary to elucidate the regulatory role of apoptosis in host immune responses using a relevant animal model, since deregulated innate immune responses reportedly contribute to severe pathogenesis due to the highly pathogenic H5N1 virus [61].

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