Institut Pasteur
Microbes and Infection 20 (2018) 65–80
www.elsevier.com/locate/micinf

Review

Apoptosis during arenavirus infection: mechanisms and evasion strategies

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Received 22 August 2017; accepted 16 October 2017
Available online 28 October 2017

Abstract

In recent years there has been a greatly increased interest in the interactions of arenaviruses with the apoptotic machinery, and particularly the extent to which these interactions may be an important contributor to pathogenesis. Here we summarize the current state of our knowledge on this subject and address the potential for interplay with other immunological mechanisms known to be regulated by these viruses. We also compare and contrast what is known for arenavirus-induced apoptosis with observations from other segmented hemorrhagic fever viruses.

Keywords: Arenavirus; Apoptosis; Cell death; Caspases; Nucleoprotein; Immunopathology

1. Introduction

The *Arenaviridae* family is composed of two distinct genera: *Mammarenavirus* and *Reptarenavirus* [1]. While reptarenaviruses are restricted to infecting snake species, where they cause Boid inclusion body disease, the rodent-borne mammalian arenaviruses have significant zoonotic potential. Indeed, several mammarenaviruses cause severe human infections, including hemorrhagic fever (HF). By far the most prevalent is Lassa virus (LASV), which causes an estimated 500,000 infections and around 5000 deaths in West Africa annually [2]. Other arenaviruses, namely Junín virus (JUNV), Machupo virus (MACV), Chapare virus (CHPV), Guanarito virus (GTOV), and Sabiá virus (SABV), produce clinically similar HF diseases in geographically restricted regions of South America. Currently, there is little understanding regarding what factors and/or biological properties are responsible for determining pathogenicity. Therefore, it is critical to advance our understanding of these viruses and their interactions with their host.

Arenaviruses are enveloped bi-segmented negative-sense single-stranded RNA viruses [3] and encode only four proteins: the polymerase (L), matrix protein (Z), glycoprotein (GPC), and nucleoprotein (NP). Because of this limited viral protein repertoire, each protein must be highly multifunctional in order to carry out all necessary functions required during the viral replication cycle. This is particularly well illustrated by the many accessory functions of NP and Z. These include inhibitory interactions of both proteins with the Type I interferon (IFN-I) production pathway [4–6], as well as interaction of NP with the Nuclear Factor-κB (NFκB) signaling cascade [7]. These interactions with the innate immune system have already been reviewed elsewhere in detail [6,8]. However, host cells have other mechanisms by which they can manage virus infection, including the ability to induce their own death if the cell becomes damaged or stressed in response to infection by pathogens. This mechanism of programmed cell death, known as apoptosis, is widely triggered in response to virus infections to limit the spread of the infection and aid in the clearance of the virus from the host [9,10]. Not surprisingly, some viruses have developed mechanisms to circumvent these pathways or even use them to their advantage. Not only does this fine-tuned regulation of cell death have important implications for virulence and pathogenicity during infection that need to be better

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https://doi.org/10.1016/j.micinf.2017.10.002

understood, but also provides possible targets to exploit for therapeutic intervention. Here we review the current state of our knowledge regarding the interaction of arenaviruses with this important cell death pathway.

2. Apoptosis

There are numerous mechanisms that can lead to cell death during development or in response to stress, damage or danger signals (reviewed in Refs. [11,12]). These include apoptosis (non-inflammatory caspase-mediated cell death characterized by cell shrinking), necroptosis (triggered by apoptotic stimuli under conditions where caspases are inhibited), caspase-independent cell death (resulting from mitochondrial membrane permeabilization but without caspase involvement), autophagic cell death (accompanied by degradation of cell components in autophagic vacuoles), oncosis (associated with ATP-depletion and cell swelling), and pyroptosis (pro-inflammatory caspase-dependent cell death). However, importantly, experimental observations are not always consistent with these clear-cut delineations, while at the same time the variety of recognized cell death processes is continuing to grow. Among currently recognized cell death processes, apoptosis is by far the most extensively studied. Apoptosis is an energy-dependent process leading to self-destruction of the cell and is characterized by formation of apoptotic bodies (via membrane blebbing), cell shrinkage, chromatin condensation, and DNA fragmentation [9]. The major mechanistic hallmark of apoptosis is the activation of caspases, which are cysteine proteases that mediate proteolytic cleavage following an aspartate residue. Caspases associated with apoptosis can be divided into initiator and executioner caspases. Initiator caspases include caspase-8, -9, and -10 and can be activated by various signaling pathways responsible for triggering apoptosis. They are then subsequently responsible for the activation of the executioner caspases, including caspase-3 and -7, which are primarily responsible for proteolytic degradation of the cell contents. At present there are over 1000 different known substrates of caspase cleavage [13] and, as a result, caspase activation leads to cell death.

There are two major pathways that result in activation of apoptotic caspases, commonly referred to as the extrinsic and intrinsic pathways (Fig. 1). The extrinsic pathway is a classic ligand-receptor pathway, where ligands such as Fas ligand (FasL), Tumor Necrosis Factor (TNF)-α or TNF-Related Apoptosis Inducing Ligand (TRAIL), bind to their corresponding receptors (i.e. Fas receptor (Fas-R), TRAIL receptor (TRAIL-R) or TNF receptor 1 (TNFR1)), so-called "death receptors", on the cell surface. This activates formation of the death-inducing signaling complex (DISC), resulting in the autocatalytic cleavage of initiator caspases-8 or -10, followed by activation of effector caspases-3 or -7. Conversely, the intrinsic pathway is initiated by a wide variety of intracellular "stress signals", such as ER-stress, reactive oxygen species (ROS) generation, DNA damage or lack of growth factors. These cellular stresses are relayed either directly or through "master apoptosis regulators" such as p53, phosphoinositide 3-kinase (PI3K) or promyelocytic leukemia protein (PML), to members of the Bcl-2 family of proteins. These proteins are characterized by the presence of one or more Bcl-2 homology (BH) domains (i.e. BH1, BH2, BH3, and BH4) and can be either pro- or anti-apoptotic in nature. In particular, many signals result in activation of the so-called BH3-only proteins, such as Bad, Bim, Bid, Bik, Puma or Noxa, which can be activated by various mechanisms including upregulation of expression, activating cleavage or phosphorylation (Fig. 1). These BH3-only proteins then either directly activate the pro-apoptotic Bcl-2 proteins Bak and Bax or inhibit the activity of anti-apoptotic Bcl-2 proteins, like Bcl-2, Bcl-x_L or Mcl1. The resulting change in the balance of activity between these pro- and anti-apoptotic Bcl-2 proteins leads to oligomerization of Bax and/or Bak, which form pores, known as mitochondrial apoptosis-induced channels, in the mitochondrial outer membrane in addition to stimulating opening of the mitochondrial voltage-dependent anion channel. This results in loss of the mitochondrial membrane potential (MMP) and release of further apoptotic factors, such as the caspase-independent apoptosis-inducing factor 1 (AIF-1) and cytochrome *c*. Together with APAF1, cytochrome *c* forms the apoptosome, which recruits and activates the initiator caspase-9, which, in turn, then activates the effector caspases-3 or -7. Importantly, while generally thought of as distinct pathways, cross-talk exists between the intrinsic and extrinsic apoptotic signaling cascades, particularly via the BH3-only factor Bid, which following cleavage and activation by caspase-8 or -10 signals via the mitochondria.

Virus infection generates many of the stress signals recognized by these apoptosis pathways, including the production of ROS during virus replication and changes in Ca^{2+} signaling during trafficking, as well as generation of pathogen-associated molecular patterns (PAMPs) recognized by cellular recognition receptors (PRRs) capable of cross-activating apoptotic pathways (Fig. 1) [6,7]. Thus, in many cases, viruses must interfere with apoptotic pathways to prevent premature cell death and prolong cellular activity in order to facilitate continued viral replication. Somewhat counterintuitively, however, certain viruses induce, rather than inhibit, apoptosis in order to facilitate their lifecycle. Therefore, depending on the virus, or, indeed, the particular stage of the virus lifecycle, cell viability must be carefully managed and a balance must be achieved between triggering apoptosis, whether intentionally or unintentionally, and promoting efficient virus replication.

3. Regulation of apoptosis during arenavirus infection

3.1. *Pathological observations* in vivo

Much of our understanding regarding the role of cell death during arenavirus infection is derived from patient autopsies and animal model necropsies. These reports give the best indications of the location, severity and implications of cell death during infection. Such autopsy analyses have revealed that, for a wide range of arenaviruses, the most severe