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Cell Death By Bacterial Porins: Evasion of Host Immunity

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Abstract
Porins are a class of outer membrane proteins which are harboured inside the outer membrane of gram negative bacteria. They are diffusion channels that are involved in transport of solutes across the outer membrane. However, their role in survival of the pathogens has been well emphasized in several reviews. Among various advantages that they confer upon the bacterium that harbours them, an important survival strategy is to induce cell death of the target host cells. They use multiple cell death mechanisms reflecting on the flexibility and evolution of their characteristics pertaining to spread of infection inside the host. In this chapter, we intend to discuss the basics of programmed cell death followed by a comprehensive account of different strategies used by porins form multiple pathogens to induce death of the infected cells.

1. Cell death

Every living organism needs to maintain a state of homeostasis in order to cope up with the extrinsic environmental changes. Cell death is one of the major processes in multi-cellular organisms that helps in achieving a balanced state inside the organism. It participates in development, control of immune responses and even controlling cancer in higher organisms (1,2). Broadly, cell death is classified into two forms; apoptosis and necrosis. Where on one hand, necrosis is an abrupt process characterized by rapid swelling and rupture of the cell undergoing it (3), on the other hand, apoptosis is a highly regulated form of cell death which is characterized by energy driven processes working in a sequence one after the other (4). Therefore, apoptosis is also referred to as programmed cell death (PCD). Until upto late 1990's, terms, apoptosis and PCD were used exchangeably. However, many reports have appeared since then suggesting that many alternative pathways of PCD occur in response to different stimuli (5,6). In a cell undergoing death, multiple PCD pathways can be activated at the same time. Therefore, PCD is a much broader term which is now used to define any form of cell death that is executed in a programmed fashion (2). Various types of cell death processes differ from each other in terms of nuclear morphology, organelles involved in the process, proteins and enzymes involved and so on. Although a universal classification for PCD has not been established till now, but based on all the above described differences, PCD can be classified into apoptosis, apoptosis-like PCD, necrosis-like PCD, necroptosis, paraptosis, pyroptosis, mitotic catastrophe and autophagy (1,2,7-10).

Apoptosis

Apoptosis is the most well established form of PCD, especially in mammalian cells. The first ever report that talked about apoptosis and introduced the term was published in

1972 by Kerr *et al* (4). Ever since then, clear markers of apoptosis along with well studied pathways have been reported in multiple research articles. An array of proteins has been reported to participate in different pathways of apoptosis, although many links are still to be discovered. Apoptosis is accompanied by massive energy driven morphological and biochemical changes in a cell undergoing the process (1,11). A cell undergoing apoptosis exhibits distinct features that can be analyzed by multiple assays. During early stages, an apoptotic cell displays cell shrinkage along with plasma membrane blebbing. Cytoplasm of an apoptotic cell becomes dense and most importantly it undergoes pyknosis due to chromatin condensation. At later stages, cellular DNA breaks into 180-200 base pair fragments followed by formation of apoptotic bodies which are finally phagocytosed by macrophages (1,11).

Apoptosis plays a crucial role in maintaining various vital processes starting from embryonic development, regulation of immune system, eradication of diseased cells to prevention of cancer (12). Not only the internal factors, but many extrinsic stimuli like chemicals, radiation, heat, hypoxia etc. can also induce apoptosis (2). Many pathogens use apoptosis as a mode of spreading infection in the host (13,14). Therefore, it is an important area of study.

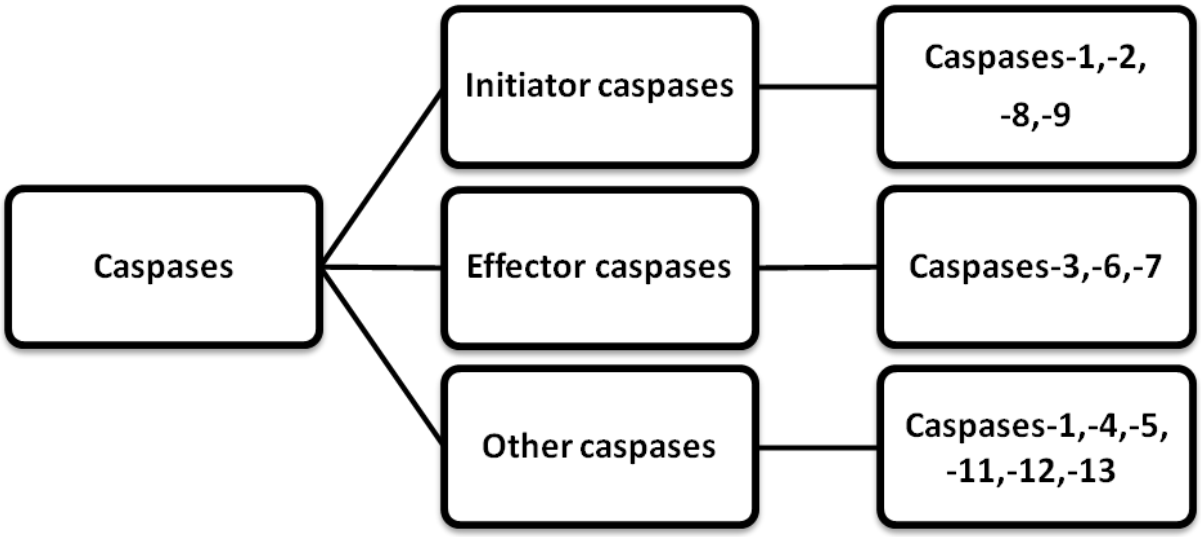
One of the major defining features of apoptosis is the involvement of a special class of enzymes called cysteine-dependent aspartate specific proteases or 'caspases' (15). First indication towards presence of these enzymes in mammals was from the existence of their structurally similar counterpart called *Caenorhabditis elegans* cell death protein, CED-3. It is involved in apoptosis of *C. elegans*. Therefore, it was thought that caspases could also be involved in apoptotic cell death in mammals (16,17). Caspases are constitutively expressed as inactive pro-enzymes called procaspases inside the cell. In activated form, all these enzymes cleave at aspartate residues of proteins but their specificity depends on the neighbouring residues in a particular protein. Once activated, they undergo autocatalytic activation, followed by activation of other caspases thereby initiating a cascade (18,19). Caspases are usually divided into three categories:

a) **Initiator caspases:** The initiator caspases are the enzymes that participate in the initial stages of major apoptotic pathways. However, initiator caspases involved in different pathways differ from each other. Each enzyme is specific to a particular pathway of apoptosis. Caspases categorized as initiators are capase-1,-2,-8,-9 (20,21).

b) **Effector caspases:** Effector or executioner caspases are caspase-3,-6,-7. These enzymes are activated by initiator caspases which once activated, lead to cell death execution (20,21). Caspase-3 is the main executioner of apoptosis (22). Upon activation, it cleaves its substrate called inhibitor of caspase-activated DNase (ICAD) which is present as a complex with an endonuclease called caspase-activated DNAse (CAD). In bound form, ICAD maintains CAD in an in-active state. When caspase-3 cleaves ICAD, CAD is released and cleaves DNA leading to its fragmentation. The initiator caspases lead to the activation of same executioner caspases in all the apoptotic pathways. Therefore, the execution of apoptosis is mediated by same set of enzymes irrespective of the initial pathway (13,23,24).

c) **Inflammatory caspases and other caspases:** Certain other caspases that do not participate in classical apoptosis may be involved in other forms of cell death. Caspases-1,-4,-5 are referred to as inflammatory caspases since they are activated at the time of inflammatory responses (20). Caspase-1 participates in a special from of inflammatory cell death called pyroptosis (10). Caspase-11 is another caspase that is

reported to participate in apoptosis and is also known to be activated at the time of septic shock. Caspase-12 is activated during endoplasmic reticulum-mediated apoptosis (21,25,26).



Pictorial representation of caspase classification.

Apoptosis mainly involves two pathways; the extrinsic pathway and the intrinsic pathway. The extrinsic pathway involves the interaction of a cell surface receptor with a ligand which further triggers the activation of a number of molecules followed by enzymes leading to cell death (27). The intrinsic pathway of apoptosis stems from mitochondria. It does not involve any receptor ligand interaction, rather the stimuli triggering intrinsic pathway initiate intracellular signals that affect mitochondria. MMP is altered and a number of pro-apoptotic molecules are released into the cytoplasm leading to apoptosis (27,28). Although the origin and initial stages of both the pathways differ from each other, final stages of execution involve the same set of enzymes.

Extrinsic pathway: Extrinsic pathway of apoptosis is mediated by activation of death receptors present on the cell surface (1,27). These receptors belong to TNF receptor super-family and are involved in multiple other functions like regulation of immune system and differentiation along with their role in cell death and survival. TNF receptor super-family involves 20 or more proteins and all of them have extracellular domains rich in cysteine (27). The characteristic feature of all the members that confers upon them the ability to transmit death signal from the surface to intracellular signalling molecules is the presence of a cytoplasmic death domain. The most well studied death receptor-ligand pairs are TNF receptor 1(TNFR1)-TNFα, TNF-related apoptosis-inducing ligand-receptor 1 (TRAIL-R1)-TRAIL, CD95 (APO/FAS)-CD95L (27,29-31).

Upon binding of ligands to their corresponding death receptors, there is induction of death receptor trimerization. As a result, the three death domains cluster together and lead to the recruitment of adaptor molecules like TNF receptor-associated death domain (TRADD) or Fas-associated death domain (FADD) (27,31). These adaptor molecules in turn recruit procaspase-8 to the activated receptor. All these components together form death inducing signalling complex (DISC). Procaspse-8 is cleaved into activated caspase due its autocatalytic activity. Activated caspase-8 further activates down-stream caspases which execute final stages of apoptosis (27,29).

An important regulator of extrinsic pathway is the FLIP protein which has two splice variants that bear homology with caspase-8 or caspase-10. Therefore, at the time of DISC formation, they might compete with procaspase-8 or procspase-10 and block their

activation. This phenomenon is exploited by some tumor cells and therefore, they become resistant to drugs or chemotherapy (32).

Intrinsic pathway of apoptosis: The intrinsic pathway of apoptosis could also be referred to as mitochondrial pathway of apoptosis as it involves major mitochondrial changes (33). Many pro-apoptotic stimuli like cytotoxic drugs and pathogen-derived molecules induce changes in the cell activating certain pro-apoptotic factors that can either permeabilize mitochondrial outer membrane or can alter the normal bio-energetic state of mitochondria (2,33,34). The latter can lead to the disruption of MMP; the electrochemical gradient across inner mitochondrial membrane (28). Loss of MMP leads to the opening of permeability transition pore (PTP). As a result, mitochondrial inter-membrane space proteins, many of which are implicated in apoptosis or even other forms of cell death are released into the cytoplasm. Mitochondrial inter-membrane space molecules involved in apoptosis are cytochrome c, Omi/HtraA2 and Smac/DIABLO (27,35).

Cytochrome c is the key mediator of intrinsic pathway of apoptosis. Once released into the cytoplasm, it interacts with apoptotic protease activating factor 1 (Apaf 1) and procaspase-9 in the presence of dATP to form a complex called apoptosome (36-38). Apaf 1 is comprised of an N-terminal caspase recruitment domain (CARD) and binding of cytochrome c to it exposes this domain by promoting association of Apapf 1 with dATP (39). The exposed domain then acts as a platform for binding of procaspase-9. Upon binding, procaspase-9 is activated and this further activates the executioner caspases leading to cell death (40).

Mitochondrial pathway of apoptosis is mainly regulated by an important class of pro-apoptotic molecules that belong to BCl-2 family of proteins (12,41). Pro-apoptotic members of BCl-2 family, like Bax, Bak, Bid or Bim can induce permeabilization of mitochondrial outer membrane and lead to the release of mitochondrial inter-membrane space proteins (42). Anti-apoptotic molecule, BCl-2, is known to prevent apoptosis. Studies have shown that over expression of BCl-2 in cells can prevent the cell from undergoing apoptosis by preventing pro-apoptotic mitochondrial changes (43).

Other mitochondrial inter-membrane space molecules like Smac/DIABLO and Omi/HtrA2 participate in intrinsic pathway of apoptosis by acting as agonists for inhibitors of apoptosis protein (IAPs) (27).

In the process of evolution, pathogenic bacteria developed new strategies to exploit host machinery for their own survival and replication (14). Different kinds of bacterial molecules are able to manipulate host-cellular responses, ranging from modulation of immune system to induction of host-cell death (14,44,45). Host-cell demise enables the pathogens to disseminate and spread inside the host (14). However, cell death is one of the major defense mechanisms activated in the host in response to bacterial infections. Induction of cell death can prevent the infection by eradicating the infected cells. Therefore, cell death responses play a crucial role during host-pathogen interactions because they can be exploited by both the opponents to their own advantage (14,45).

2. Cell death by Porins

Many gram-negative bacteria have been studied for their ability to manipulate signaling mechanisms involved in host-cell death. Pathogens like *Salmonella*, *Shigella*, *Yersenia*, *Helicobacter pylori*, enteropathogenic *E. coli*, *Neisseria*, *Pseudomonas aeruginosa* and many more are known to induce or suppress different mechanisms of PCD in different types of host cells (14,45-47). The molecules employed by these bacteria to execute or prevent cell death could be their structural components, toxins, secretion factors or even outer membrane channel proteins called porins (45,48,49). For example, *Helicobacter pylori* uses its factor CagA to inhibit apoptosis by up-regulation of anti-apoptotic proteins like Mcl-1(50). Similarly, factors NleH and NleD of enteropathogenic *E. coli* prevent apoptosis by

inhibiting caspase-3 activation (51,52). Flagellin of *Salmonella enterica* Typhimurium induces pyroptosis in host cells (53,54). *Shigella flexneri* induces host-cell necrosis through mitochondrial dysfunction (55). *Yersinia* induces apoptosis in macrophages through translocation of its effector protein, YopJ (56,57). *Nesseria meningitides* is known to inhibit apoptosis in epithelial cells through its porin which interacts with the mitochondria and prevents MMPT (58,59). However, porin from another species of *Neisseria* i.e. *Neisseria gonorrhoeae* is known to induce apoptosis in target cells by being translocated to host-cell mitochondria (60). Porin from *Pseuodomonas aeruginosa* is also known to induce apoptosis through calcium influx (61).

Porins from gram-negative bacteria are well established for their contribution in virulence and pathogenesis of the bacteria harbouring them. Pathogenic gram-negative bacteria have developed multiple ways of using their porins to enhance virulence (62,63). Porins are known to help in the invasion process of bacteria, attachment and colonization inside the host, survival inside the host, inducing host-cell death and in eliciting immune responses (44,61,64-66). Location of porins on the bacterial outer membrane makes them easily accessible to complement molecules inside the host. Also, their presence in the outer membrane facilitates their secretion in vesicles released by gram-negative bacteria (62). Another major reason for the pathogenic role of porins could be their similarities with mitochondrial porins (67,68). Due to this characteristic, porins can disrupt the ion composition of the infected cells (62). Moreover, it has been suggested that PorB from *Nesseria gonorrhoeae* specifically targets mitochondria and behaves like mitochondrial porin VDAC (66,69). This feature has been attributed to the endosymbiotic origin of mitochondria which enables the bacterial porins to exploit the similarities between bacterial membrane and mitochondrial membrane (69). Owing to the array of functions that porins can perform, functional studies regarding different roles of porins are important. Various studies have been done in this regard along with the cancer cells and successful findings have been achieved [70-79].

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