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BACTERIA-INDUCED APOPTOSIS: AN APPROACH TO BACTERIAL PATHOGENESIS

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ABSTRACT

Several pathogenic or opportunistic bacteria can induce or inhibit host cell apoptosis. The modulation of cellular pathways that results in the induction or delay of host cell apoptosis is an important mechanism of bacterial virulence. These processes can be mediated by various host cell signaling pathways that are subverted by the bacteria. Pathogens can activate apoptotic proteins such as caspases, inactivate anti-apoptotic proteins such as NFκB and mitogen-activated protein kinases, or up-regulate the endogenous receptor/ligand system that induces apoptosis, generally when the bacteria are bound to the host cell surface. Bacteria-induced apoptotic or anti-apoptotic processes are often related to the ability of the bacteria to reach the host tissues. However, since apoptosis is also involved in host defense mechanisms against infectious agents, this phenomenon apparently plays a central role in host-pathogen interactions.

Key words: Apoptosis, bacteria, bacteria-induced apoptosis, pathogenicity, virulence

INTRODUCTION

Apoptosis is defined as cell death activated by an internally controlled suicide program and involves a subtly orchestrated disassembly of cellular components designed to eliminate unwanted cells in various physiological processes during embryogenesis. During apoptosis, doomed cells are removed with minimum disruption to the surrounding tissue. However, apoptosis also occurs under pathological conditions and is sometimes accompanied by necrosis [24]. Bacterial infections in particular play an important role in triggering apoptosis.

The bacteria that use apoptotic mechanisms include a variety of facultative intracellular pathogens, such as *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Salmonella* spp., *Shigella* spp., *Haemophilus influenzae*, *Neisseria meningitides* and *Neisseria gonorrhoeae*, as well as bacteria that are not considered typical facultative intracellular pathogens, such as *Helicobacter pylori*. Strictly intracellular pathogens such as the genera *Rickettsia* and *Chlamydia* can also cause apoptosis. In this review, we discuss the main mechanisms used by strictly and facultative intracellular bacteria to induce apoptosis, and the relationship between this phenomenon, virulence and pathogenicity. The molecular characterization of apoptosis and the interactions between host and bacterial cells are also considered.

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Molecular mechanisms of apoptosis

Bacteria can trigger apoptosis through a large variety of mechanisms that include the secretion of protein synthesis inhibitors, pore forming proteins, molecules responsible for the activation of the endogenous death machinery in infected cells, and super antigens. Since many of the enzymes and signal transduction pathways that mediate apoptosis have been extensively discussed in several recent reviews [51,61,77,78,84,95,111], we will restrict this review to molecules induced by bacteria involved in apoptosis. The first group of enzymes involved in many forms of apoptosis, including bacteria-induced apoptosis, are the caspases [95]. These host cell cytoplasmic proteases cleave many cellular proteins to produce alterations in membrane symmetry, mitochondrial function, and DNA fragmentation. Mitochondria play a key role in apoptosis [58,97] since they depolarize, swell, and release pro-apoptotic factors during this process. The

pro-apoptotic factors include cytochrome c and the apoptosis inducing factor (AIF) [61]. Cytochrome c associates with a scaffold protein APAF-1 to activate caspase 3 and produce cell death [111]. AIF can trigger cell death independently of caspases by being translocated directly to the nucleus to induce DNA fragmentation [51,53]. In addition, CD95 or TNF-receptor uses the activation of caspase 8 to trigger downstream events such as mitochondrial changes and/or caspase 3 activation, whereas primary DNA damage apparently mediates apoptosis primarily via mitochondria, followed by the activation of "execution" caspases. Pro-apoptotic molecules are balanced by anti-apoptotic factors, with the "inhibitors of apoptosis" (IAPs) and Bcl-2-like proteins being some of the most important anti-apoptotic factors. IAPs can associate with and directly inhibit caspases [108], but regulation of these proteins by bacterial factors has not yet been demonstrated. Bcl-2-like proteins constitute a family of pro- and anti-apoptotic proteins. The Bcl-2 factor, Bcl-xL, and other members prevent apoptosis induced by many stimuli, whereas Bim, Bax, Bad, and other members promote apoptosis [78,84,106]. The mode of action of Bcl-2-like proteins is still unclear.

The mechanisms of bacteria-induced apoptosis that use the host machinery will be discussed with reference to *Shigella flexneri*, *Salmonella typhimurium*, *Yersinia enterocolitica*, *Y. pestis*, *Y. pseudotuberculosis* and *Pseudomonas aeruginosa.* Although many other bacteria, including enteropathogenic *Escherichia coli*, *Listeria monocytogenes* and *Neisseriae,* also trigger apoptosis, we will limit our considerations to a few bacteria to demonstrate the paradigms of bacteria-induced activation of host cell apoptosis (Fig. 1).

Bacteria-induced apoptosis

Shigella and *Salmonella*

Historically, the genus *Shigella* consists of four pathogenic "species": *S. dysenteriae*, *S. flexneri*, *S. sonnei,* and *S. boydii*. However, DNA hybridization experiments have clearly established that this genus consists of a single species that also includes *Escherichia coli*, or vice-versa [89]. In this review, we will use the former nomenclature. *Shigella flexneri*, the causative agent of bacillary dysentery, induces apoptosis in macrophages *in vitro* and *in vivo* [48,53,113,115]. In this process, bacterial internalization and subsequent escape into the cytosol are essential for pathogenesis. In the cytosol, *S. flexneri* translocates the plasmid-encoded invasion antigen B (IpaB) via a type-III secretion system. IpaB directly binds and activates caspase-1, and its translocation results in apoptosis [45]. The induction of apoptosis by *Shigella* is tissue-specific and this bacterium does not induce apoptosis in epithelial cells [64].

An intriguing model has been proposed for the role of apoptosis in controlling *Shigella* infection [112]. In this model, caspase-1 plays dual pivotal roles in driving macrophage apoptosis and acute inflammation. Caspase-1 activation and the apoptotic death of *Shigella*-infected macrophages cause the release of mature interleukin IL-1β [114], which in turn recruits polymorphonuclear leukocytes (PMNs) to the infection sites [112]. The PMNs cross the intestinal epithelium, altering the integrity of this epithelial barrier. This promotes massive secondary invasion of the bacteria and acute inflammation [112]. The roles of apoptosis and the release of mature IL-1β in the pathogenesis of shigellosis have also been demonstrated *ex vivo* in a rabbit-ligated-loop infection model of experimental shigellosis [8,93]. Furthermore, studies *in vivo* of cytokine production by cells of patients with shigellosis have shown that the number of TNFα-producing cells is increased, suggesting that TNFα may play a role in activating caspase-1 and the subsequent apoptotic pathway in the host cell *in vivo* [83]. This model for the role of apoptosis in the pathogenesis of bacterial infections could have implications for acute infections caused by several other intracellular bacteria, such as *Salmonella* and *Listeria*.

A molecular interaction identical to that of *Shigella* is used by *Salmonella,* which also induces apoptosis in macrophages [104]. Using a type III secretion system, intracellular *Salmonella* secretes an IpaB homolog (SipB) into the cytosol, where it directly binds to and activates caspase-1 [44]. In mice, there is a correlation between *Salmonella*-induced apoptosis of macrophages and disease progression, suggesting that apoptosis may play a role in this infection [85]. *Salmonella*-induced apoptosis contributes to the escape of intracellular bacteria from spent host cells following nutrient deprivation and the termination of bacterial replication. This is because *Salmonella*-induced macrophage apoptosis is enhanced after transition from the logarithmic to the stationary phase growth [62].

Although *Salmonella* causes an acute localized inflammation in the intestine that is similar to the acute infection caused by *Shigella*, this genus can also potentiate fatal systemic infections not commonly associated with shigellosis [49,93]. Hence, similar mechanisms for inducing apoptosis are used by *Shigella* and *Salmonella,* and the role of apoptosis in modulating the pathogenesis of the disease is probably similar in some (but not all) aspects. For example, *Salmonella*-induced apoptosis might confer acute gastroenteritis, similar to that associated with shigellosis, which eventually controls the infection. Alternatively, modulation of the host apoptotic pathways could cause down-regulation of the host immune response, resulting in systemic spread of the pathogen, as in the case of salmonellosis. This is reminiscent of the bacterial dissemination and systemic infection caused by another pathogen, *Yersinia*, in which the induction of apoptosis has been suggested to play a role in the pathogenesis of infection [18,71].

Like *Shigella*, *Salmonella enterica serovar typhimurium* also does not induce apoptosis in epithelial cells [62]. This may be because of a true inability to induce apoptosis in intestinal epithelial cells or because of blockade by *Shigella* because these intestinal cells are the primary sites for intracellular bacterial proliferation during shigellosis. Hence, epithelial cells are an important compartment for bacteria because they enable these pathogens to cause disease [113,115].

Escherichia coli

The apoptotic episode caused by *E. coli* has been associated mainly with the ability of some *E. coli* (STEC) to produce a Shiga-like toxin, including the most common serotype (O157:H7). STEC is thus a bacterial enteropathogen capable of binding to the intestinal epithelium and producing Shiga-like toxins, which are associated with hemorrhagic colitis and the hemolytic-uremic syndrome in humans [5,82]. The apoptosis of intestinal epithelial cells decreases barrier functions [1] and could provide a mechanism for Shiga-like toxins to enter the bloodstream [47].

As discussed above, caspases play an central role in mediating the intracellular signaling events that result in apoptosis [22]. Caspase-8 is involved in the Shiga-like toxin-mediated apoptosis of epithelial cells. However, whether the Shiga-like toxin activates caspase-8 directly via Gb3 binding or indirectly through death receptors and ligands remains to be elucidated. The activation of caspase-8 probably results in the induction of cytochrome c release from the mitochondria, thereby activating procaspase-3, which in turn activates caspase-9 and the mitochondria death pathway [59,63]. Knowledge of the molecular pathogenesis of enterotoxigenic *E. coli* infection could provide the basis for the development of novel treatment strategies that would interrupt progress of the disease. Caspases are potential and promising therapeutic targets for the modulation of apoptosis. For instance, treatment with a caspase inhibitor prevented cell death and reduced neurological damage in an animal model of bacterial meningitis [15].

Yersinia

Yersinia invades several types of mammalian cells *in vitro*, including epithelial cells and fibroblasts, and M cells *in vivo*. However, several *Yersinia*-secreted proteins block internalization by professional phagocytes. *Yersinia* induces apoptosis in macrophages *in vitro* [69,72,90] and *in vivo* [71]. Translocation of the effector molecule YopJ (*Yersinia pseudotuberculosis*) or YopP (*Yersinia enterocolitica*) into the macrophage by the type III secretion system is required for the induction of apoptosis. *Yersinia* YopJ/P represses activation of the nuclear factor NFκB by inhibiting the phosphorylation and subsequent degradation of its inhibitor protein, IκB. As a result, the apoptotic process and the production of TNFα by *Yersinia*-infected cells is repressed [69,94]. YopJ/P binds directly to proteins of the mitogen-activated protein kinase (MAPK kinases) superfamily, blocking both phosphorylation and activation. The inhibition of MAPKK activity by YopJ/P explains how this single bacterial factor blocks several signaling pathways regulated by JUNK, p38, and also NFκB signaling, thereby preventing cytokine synthesis and promoting apoptosis [80]. The ability of *Yersinia* to eliminate phagocytic cells by apoptosis and to down-regulate inflammatory cytokines undoubtedly promotes bacterial dissemination.

The role played by the anti-apoptotic MAPKKs and NFκB pathways of the infection by *Yersinia* in macrophages has recently been elucidated. Both MAPKKs and NFκB up-regulate apoptosis in response to the infection [110]. Also, the Toll-like receptor 4 (TLR4) [81] has been shown to act as a potent inducer of apoptosis in macrophages [41].

This cellular receptor is implicated in starting and regulating the apoptotic process, together with MAPKK and NFκB, whereas different intracellular factors are controlled by YopJ/P or other pro-apoptotic proteins.

Pseudomonas aeruginosa

A distinct mechanism is used by *Pseudomomas aeruginosa* to trigger the cell death of infected host epithelial cells. *P. aeruginosa* is considered to be one of the most important bacteria in clinical practice because it is resistant to many antibiotics and plays a crucial role in life-threatening infections in immunocompromised patients. Of particular importance are pulmonary infections by *P. aeruginosa* in patients with cystic fibrosis. Almost all patients with this disease develop chronic *P. aeruginosa* infections that cause lung destruction and pre-mature death of the patient.

Upon infection of epithelial cells *in vitro* or *in vivo, P. aeruginosa* induces up-regulation of the CD95/CD95 ligand on the cell surface [39]. The CD95/CD95 ligand system is one of the most important endogenous receptor ligand pairs triggering apoptosis. The up-regulation of CD95 and the CD95 ligand on cells infected with *P. aeruginosa* depends on the function of the type III secretion system. When the bacteria lack this secretion system, they almost fail to trigger apoptosis in epithelial cells. The binding of CD95 by the CD95 ligand upon up-regulation induces the activation of caspases 8 and 3, the release of mitochondrial cytochrome c, and JUNK activation. Furthermore, reactive oxygen intermediates seem to be important in the induction of *P. aeruginosa*-triggered death [99]. The significance of the CD95/CD95 ligand system for *P. aeruginosa*-triggered cell death is shown by genetic studies using cells or mice genetically deficient in functional CD95 or CD95 ligand. Epithelial cells obtained from CD95- or CD95 ligand-deficient mice or fibroblasts lacking either CD95 or the CD95 ligand did not undergo apoptosis in response to infection by *P. aeruginosa*.

Helicobacter pylori

Helicobacter pylori can mimic the apoptotic mechanism by producing the pro-apoptotic toxin VacA. This toxin is rapidly transported into the mitochondria of epithelial cells and induces changes consistent with the permeabilization of mitochondrial membranes. This damage involves a mechanism that requires cellular entry dependent on a toxin with membrane channel activity. Targeting of the mitochondrial membranes is a strategy used by pathogenic microbes to control cell viability while circumventing upstream pathways and checkpoints associated with cell death. Persistent *H. pylori* infections in the human gastric mucosa are a significant risk factor for the development of gastric and duodenal ulcers, as well as stomach cancer. The rate of infection in humans is greater than the incidence of *H. pylori*-mediated diseases, the onset of which is influenced by multiple factors, including the virulence of the infecting strain and the genetic predisposition of the host. Apoptosis within the gastric mucosa is strongly associated with the presence of *H. pylori* [50,91], which induces apoptosis in *in vitro* in human gastric adenocarcinoma cells, as well as in murine and gerbil models of infection [9,103]. Although multiple *H. pylori* factors have been reported to be pro-apoptotic, VacA was recently demonstrated to be sufficient to induce cellular apoptosis [13,25]. The pro-apoptotic activity of VacA can serve multiple functions during infection by *H. pylori*, including colonization of the stomach by killing the parietal cells responsible for maintaining the acidic environment [92].

Neisseria

The pathogenesis of *Neisseria meningitidis* and *N. gonorrhoeae* requires their interaction with human cells and cellular barriers. These pathogens interact with blood, plasma, and exudate fluids, and also adhere to and invade epithelial and endothelial cells [66,68,100-102]. Several *Neisseria* components are involved in the modulation of pathogen-host cell interactions, including type IV pili, Opa proteins and porins. There is conflicting information regarding the effects of neisserial porins on apoptosis. Muller *et al.* [73-75] demonstrated that the *N. gonorrhoeae* porin PorB1B interacts with HeLa cell mitochondria and induces calcium efflux and apoptosis. However, meningococcal PorB can protect against mitochondrial apoptosis induced by staurosporine [28,65].

There are several explanations for the divergent effects of *N. gonorrhoeae* PorB1B and *N. meningitidis* PorB in apoptosis. These include structural variations in the two porins, differences in the procedures used to purify these proteins, differences in the cell culture conditions (especially the absence [73-76] or presence

[65] of fetal calf serum in the culture medium), and intrinsic differences between the cell lines used in the experiments. The mechanism by which neisserial porins activate lymphocytes B is probably related to their adjuvant activity, which would be attenuated if apoptosis were also induced. Hence, the inhibition of immune cell apoptosis by neisserial porins could be inherent in their immunopotentiating ability.

Since pathogenic *Neisseria* invade host cells, blocking host cell apoptosis might provide enough time for the pathogen to adapt to the new environment and multiply to sufficient levels, thereby allowing further infection. Additionally, the loss of intracellular ions, particularly potassium, plays a primary role in apoptosis, and a better understanding of the role of ion channels and plasma membrane transporters in cellular signaling during apoptosis could have important physiological implications for lymphocyte function. This information could be important for the design of therapeutic strategies for several diseases of the immune system in which apoptosis is involved.

Another mechanism also involved in *Neisseria*-mediated apoptosis is the meningococcal lipooligosaccharide (LOS) present in the outer bacterial membrane and that is released as vesicles or 'blebs' from surplus outer membrane material [27]. Native outer membrane vesicles (OMVs) contain 24-50% LOS relative to the protein [12], whereas OMVs purified from *N. meningitidis* and partially detoxified so that they can be used for vaccination contain 5-9% LOS relative to their protein content [32]. Recently, two strong candidates for the LOS signaling protein have been identified in myeloid cells and are known as Toll-like receptors 2 and 4 (TLR2 and TLR4) [6,30,55,60,96,107,109]. TLR2 is involved in the recognition of Gram-positive bacteria, *Mycobacterium* [30,109], and bacterial products such as lipopeptides [16,60], whereas TLR4 mediates apoptotic of Gram-negative bacteria by LOS or LPS [19,43] together with CD14 [105].

Legionella

The agent of Legionnaire's disease, *Legionella pneumophila*, invades and replicates within alveolar macrophages and monocytes and, possibly, alveolar epithelial cells. Studies *in vitro* have shown that *L. pneumophila* and *L. micdadei* induce apoptosis in macrophages and alveolar epithelial cells [17]. The expression of apoptosis-inducing factor(s) by *L. pneumophila* is apparently regulated by the Dot/Icm type IV-like secretion system because several *dot/icm* mutants fail to induce apoptosis [36-38]. Thus, *L. pneumophila*-induced apoptosis via cell contact might be mediated by binding of the pathogen to a common receptor on macrophages and epithelial cells, or via translocation of an effector protein through the Dot/Icm secretion system [33-38,76].

Although the induction of macrophage apoptosis by *L. pneumophila* is a constitutive event, necrosis mediated by the *Legionella* pore-forming toxin is temporarily triggered upon the termination of bacterial proliferation [33-38,76]. This could represent a coordinated strategy used by this intracellular pathogen to invade, proliferate within, and eventually exit from the spent host cell. *Legionella pneumophila*-induced apoptosis plays a role in intracellular trafficking and evasion of endocytic fusion, at least during the early stages of the infection. *L. pneumophila* completely blocks maturation of its phagosome via the endosomal-lysosomal degradation pathway [2,3,20,23].

Apoptosis induced by some facultative intracellular bacteria that block endocytic fusion (such as *Legionella*) could modulate the biogenesis of their vacuoles into idiosyncratic niches suitable for intracellular proliferation. The ability of *L. pneumophila* to proliferate intracellularly and to kill the host cell by apoptosis and necrosis seen in tissue culture does not go determined *in vivo*. A large proportion of Legionnaire's disease patients are immunocompromised, and several host immune responses are activated and are effective against infection by *L. pneumophila*. Therefore, although *L. pneumophila* is apparently an uncontrolled pathogen *in vitro*, its fate *in vivo* is controlled at complex levels, presumably by both bacterial and host effector mechanisms, such as INFγ. During the early stages of infection and exponential replication, *L. pneumophila* activates caspase-3 by a Dot/Icm-dependent process without driving the infected cell into apoptotic death. At any stage of infection, intracellular replication ceases when apoptosis is triggered in the host cell either by *L. pneumophila* or via caspase-3 activation by pharmacological agents. High caspase-3 activity is observed throughout the exponential intracellular replication of the pathogen. Caspase-3 binds to the apoptotic cell in the late stages of infection, concomitant with the termination of intracellular replication [4].

Amebas are the natural hosts of *L. pneumophila* in the environment [3]. *L. pneumophila* does not

induce apoptosis in amebas, although the latter can undergo apoptosis following proper stimulation. In contrast to the biphasic killing of mammalian cells, *L. pneumophila* kills amebas during the late stages of the infection exclusively via necrosis mediated by pore-forming toxins. This mode of killing is essential in order for the bacteria to leave the protozoan host after terminating replication [33,41,42]. *L. pneumophila* disrupts the phagosomal membrane and becomes cytoplasmic in the last stages of infection in macrophages and *Acanthamoeba polyphaga*. Lysossomal elements, mitochondria, cytoplasmic vesicles, and amorphous material are dispersed after phagosomal disruption in human macrophages and *A. polyphaga* [70].

Listeria

Listeria monocytogenes induces apoptosis by lysing the phagosomal membrane and escaping into the cytosol to initiate intracellular infection, a process that is mediated by a secreted pore-forming toxin, listeriolysin O (LlyO). *L. monocytogenes* induces LlyO-dependent apoptosis [40] in a variety of cell types, including hepatocytes [86], lymphocytes [67] and dendritic cells [40]. The insertion of LlyO into the mitochondrial membrane may cause the release of cytochrome c that in turn activates the caspase cascade. Alternatively, the insertion of LlyO into the mitochondrial and/or endoplasmic reticulum membrane may stimulate calcium efflux, thereby activating the calcium-dependent protease calpain and/or caspases [8]. In contrast to most other intracellular bacterial pathogens that induce apoptosis, *L. monocytogenes* does not induce apoptosis in macrophages but causes LlyO-mediated necrosis [11]. Despite the lack of apoptosis in macrophages and no release of mature IL-1β (an indicator of apoptosis – Figure 1), *Listeria*-infected hepatocytes produce PMN chemo-attractants during the early stages of infection [86]. These chemo-attractants are involved in the phagocytosis of dead cells such as hepatocytes. Apoptosis in hepatocytes results in hepatic abscesses that represent the first physiological barrier to *Listeria*.

Mycobacterium

Mycobacterium tuberculosis induces apoptosis in macrophages *in vitro* and *in vivo* via a TNFα- and caspase-1-dependent pathway [31,52,87,88]. TNFα production and the induction of apoptosis in macrophages are mediated by the binding of mycobacterial cell wall components and/or lipoproteins to the Toll-like receptor-2 (TLR-2) [7,98]. Other bacterial cell surface products such as *Borrelia burgdorferi* lipoproteins also bind TLR-2 and activate the human cell lines U373 and HEK 293 [16,46]. However, it is unclear whether these interactions also result in apoptosis, particularly because most pathogenic bacteria have lipoproteins. Nevertheless, some components of the mycobacterial cell wall, such as lipoarabinomannan (LAM) [14], can also influence apoptosis caused by this pathogen. LAM can activate the apoptotic cycle through phosphorylation of the Bad protein, thereby preventing binding to the anti-apoptotic proteins Bcl2 and BclX [14].

M. tuberculosis also protects cells against apoptosis via two key pathways: induction of the TLR-2-dependent activation of the NFκB cell survival pathway [7] and enhancement of the production of the soluble TNF receptor 2 (sTNFR2), which neutralizes the pro-apoptotic activity of TNFα [10,56]. Consequently, the modulation of apoptotic pathways by *M. tuberculosis* is complex and includes the induction of cell-death and cell-survival pathways. The extent to pro- and anti-apoptotic activities are manifested during different stages of the infection is unknown. The modulation of apoptosis by *M. tuberculosis* and its direct and overlapping effects on the immune system probably play key roles in pathogenesis.

Macrophage apoptosis occurs within the granuloma. This histological process favors host immunity, but *M. tuberculosis* is capable of partially suppressing it. The pro- and anti-apoptotic activities of *M. tuberculosis* may be necessary to establish a persistent infection. Vaccines and immunotherapies that result in increased levels of macrophage apoptosis within the granuloma may tip the balance away from *M. tuberculosis* virulence towards a successful host immune response. A better understanding of the signaling pathways and effector mechanism(s) triggered during apoptosis should lead to new immunotherapies that can stimulate macrophages to kill *M. tuberculosis* [14,54].

In conclusion, apoptosis induction by *M. tuberculosis* involves three main events, namely, arrest during phagosomal maturation (mediated calcium ions and the cytoplasmic protein calmodulin), the anti-apoptotic response (in which Bcl proteins are in-

volved), and suppression of the host cell antibacterial response (mediated by MAPK) [57]. *Mycobacterium* species are therefore well adapted to the hostile environment of phagocytic cells and use several survival strategies not seen in other bacteria.

Chlamydia

Chlamydia consists of intracellular bacterial species that can affect the apoptotic pathways in two opposing directions, each of which is manifested during different stages of the infection. During the early stages of infection, *Chlamydia trachomatis* protects infected cells against apoptosis induced by a wide spectrum of stimuli, including the kinase inhibitor staurosporine, the DNA-damaging agent etoposide, TNFα, an anti-FAS antibody, and granzyme B/perforin. In addition to the complete inhibition of caspase-3 activity in *Chlamydia*-infected cells, the release of cytochrome c from mitochondria is also blocked during the early stages of infection [29]. However, *Chlamydia psittaci* induces apoptosis in macrophages and epithelial cells during the late stages of infection, and this induction requires intracellular bacterial replication [79].

Although the bacterial factor(s) that trigger(s) apoptosis in the host cell is unknown, *Chlamydia* activates an apoptotic pathway that is apparently independent of known caspases, in a manner

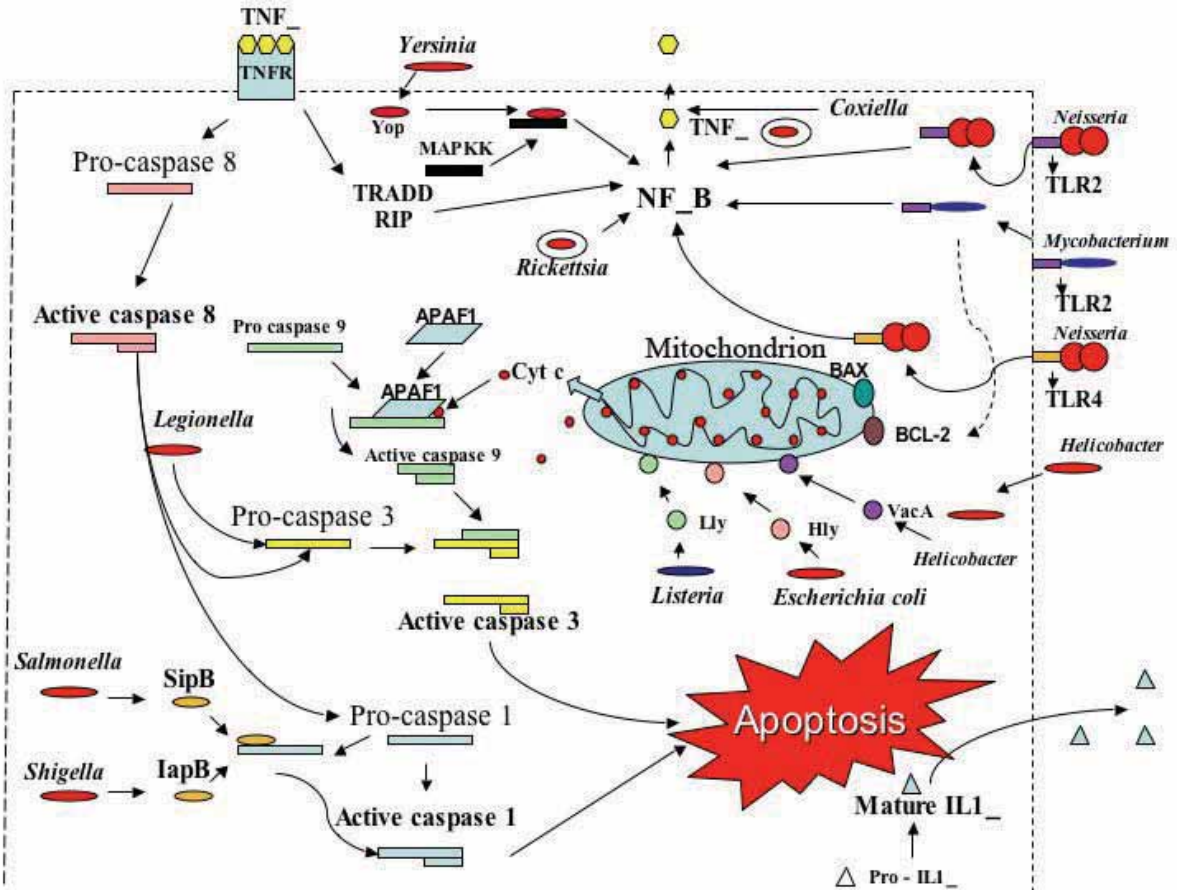

Figure 1. Main mechanisms of bacteria-induced cell apoptosis. The broken lines represent the cellular membrane that separates the extracellular and intracellular environments. The apoptotic pathways induced by *Helicobacter pylori* (via action of VacA on the mitochondria), *Neisseria* (via NFκB activation of the apoptotic mechanism), *Escherichia coli* (apoptosis initiated by the action of hemolysin- Hly on mitochondria) and pathogens normally associated with apoptotic processes such as *Listeria* (Lly action), *Yersinia* (activation of MAPKK and NFκB pathways), *Shigella*, *Salmonella* (caspase 1 activation pathway), *Legionella* (caspase 3 apoptotic activation pathway), *Mycobacterium*, as well as strictly intracellular pathogens such as *Rickettsia* and *Coxiella* (similar to the mechanism of *Neisseria*-induced cell apoptosis, see above), are shown. Modified from Gao and Abu-Kwaik [34].

reminiscent of the caspase-independent apoptosis induced by the overexpression of Bax [106]. The dual activity of *Chlamydia* in manipulating host cell apoptosis indicates a strategy by which intracellular bacteria control the balance between anti- and pro-apoptotic activities at discrete stages of the infection [79]. *Chlamydia* species require several days of intracellular replication and differentiation to produce sufficient infectious elementary bodies to spread to adjacent cells. These bacteria also rely on the host cell integrity and metabolic activities for their strict parasitic lifestyle. Hence, the anti-apoptotic activity exerted during the early stages of infection helps to maintain the metabolic activities of the infected cell [29,79]. Subsequent activation of the host cell apoptotic pathways in the late stages of infection may facilitate dispersal of the bacteria and initiate a host inflammatory response that eventually controls the infection. In addition to the role of bacterial effector molecules, the pro-apoptotic and anti-apoptotic effects of *Chlamydia* at different stages of the infection may be modulated by extracellular mediators such as cytokines.

Rickettsia and *Coxiella*

Another obligate intracellular bacterial genus, *Rickettsia,* also blocks apoptosis in the host cell. In contrast to the apoptosis induced by the inhibition of NFκB by *Yersinia*, *Rickettsia rickettsii* protects infected vascular endothelial cells from apoptosis via activation of the NFκB signaling pathway [21]. However, apoptosis of the infected cells occurs when *R. rickettsii*-induced activation of NFκB is inhibited, which suggests that *R. rickettsii* also has a pro-apoptotic activity [21]. Although the nature of this pro-apoptotic activity is unknown, the TNFα-mediated apoptotic pathway is not involved [21]. Alternatively, apoptosis could be a host cell response to bacterial invasion that is normally prevented by bacterial activation of NFκB.

The infection of macrophages by the obligate intracellular bacterium *Coxiella burnetii* stimulates the production of TNFα and TNFα-mediated apoptosis during the late stages of infection [26]. Apoptosis of infected macrophages is associated with a moderate release of IL-1β, which suggests a role for caspase-1 [26]. As in the case of *M. tuberculosis*, the apoptosis of infected macrophages is associated with a marked reduction in the viability of intracellular bacteria [26], which suggests a protective role for apoptosis in infection by *C. burnetii*. A possible anti-apoptotic activity of *C. burnetii*, especially during the early stages of infection, remains to be demonstrated.

CONCLUDING REMARKS

In this review, we have described the main differences and similarities among the mechanisms used by bacteria to induce apoptotic or anti-apoptotic effects in host cells. These mechanisms were explored by comparing the apoptotic pathways in host cells infected by obligate intracellular bacteria (*Rickettsia, Chlamydia, Coxiella*) and by facultative intracellular (*Salmonella, Shigella, Legionella, Listeria, Mycobacterium*) and extracellular (*Pseudomonas, Neisseria, Helicobacter* and *Escherichia coli*) bacteria. The main events triggered by the microorganisms discussed here are specific for each pathogen and include the activation of caspases, mitochondrial alterations, and the activation of MAPK kinases. Elucidation of the signaling pathways, the cellular receptors and/or the bacterial factors involved in the induction of apoptosis could reveal new therapeutic targets for blocking bacterial-induced apoptosis. The development of drugs towards such targets should provide us with new tools for treating several diseases caused by bacterial pathogens.

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