

## REVIEW

## Targeting apoptosis pathways in infections

Thomas Naderer | Maria Cecilia Fulcher

Biomedicine Discovery Institute and  
Department of Biochemistry & Molecular  
Biology, Monash University, Clayton, Australia

## Correspondence

Thomas Naderer, Biomedicine Discovery  
Institute and Department of Biochemistry &  
Molecular Biology, Monash University,  
19 Innovation Walk, Clayton, VIC 3800,  
Australia.  
Email: Thomas.naderer@monash.edu

## Abstract

The programmed cell death pathway of apoptosis is essential for mammalian development and immunity as it eliminates unwanted and dangerous cells. As part of the cellular immune response, apoptosis removes the replicative niche of intracellular pathogens and enables the resolution of infections. To subvert apoptosis, pathogens have evolved a diverse range of mechanisms. In some circumstances, however, pathogens express effector molecules that induce apoptotic cell death. In this review, we focus on selected host-pathogen interactions that affect apoptotic pathways. We discuss how pathogens control the fate of host cells and how this determines the outcome of infections. Finally, small molecule inhibitors that activate apoptosis in cancer cells can also induce apoptotic cell death of infected cells. This suggests that targeting host death factors to kill infected cells is a potential therapeutic option to treat infectious diseases.

## KEYWORDS

BCL-2, caspase, cell death, inflammation, *Legionella*, mitochondria

## 1 | INTRODUCTION TO THE ROLE OF APOPTOSIS IN MAMMALS

Cellular suicide plays many essential roles in mammals.<sup>1</sup> At the earliest stages of development, cell death eliminates excess cells during embryogenesis to enable finger and toe formation.<sup>2</sup> In neuronal development, cell death removes neurons that are no longer required after synaptic contacts have been made at their final targets.<sup>3,4</sup> Male fertility depends on cell death to shape the germinal epithelium within testes, which promotes maturation of haploid sperm cells.<sup>5</sup> Cellular suicide also maintains tissue homeostasis by removing spent or damaged cells and shapes the immune system by eliminating dangerous cells. Conversely, the immune system employs cell death to remove infected cells and to prevent the spread of microbial and viral pathogens. In all of these cases, cell death is highly regulated and controlled by genetic programs rather than caused accidentally. It is now evident that cells can induce many forms of programmed cell death that affect

development, immunity, and inflammation in various ways. In this review, we focus on the programmed cell death pathway of apoptosis and discuss recent findings on its role in either preventing or promoting infections. The functions of other forms of programmed cell death, such as pyroptosis and necroptosis, in infections have recently been reviewed elsewhere and in this issue.<sup>6–8</sup>

Not all cells die alike (Table 1). Inflammatory forms of cell death are associated with plasma membrane rupture, cell swelling, and the release of cytosolic content.<sup>9</sup> In contrast, apoptotic cells show distinct morphological changes including cell and organelle shrinkage due to fluctuations in ion concentrations, DNA fragmentation, and the formation of dynamic plasma membrane protrusions. Apoptosis also triggers plasma membrane blebbing rather than rupture, which results in the packaging of cytosolic content within apoptotic bodies.<sup>10</sup> Apoptotic cells and bodies release and expose so-called “find me/eat me” signals, such as nucleotides and phosphatidylserine, which enable the detection and engulfment by professional phagocytes before plasma membrane rupture.<sup>11</sup> This allows for the rapid clearance of dead cells without the spillage of cytoplasmic content into the extracellular space. The removal of dead cells is highly efficient as up to  $1 \times 10^{10}$  cells in an adult human are eliminated every day from tissues and organs to maintain health.<sup>1</sup> The failure to induce apoptosis can lead to cancer. This is evident from pioneering studies showing that increased expression of B-cell lymphoma 2 (BCL-2) renders lymphocytes refractory to cell death upon cytokine deprivation, which promotes their accumulation in mice and causes cancer.<sup>12–14</sup> BCL-2 was initially discovered from human

Abbreviations: BAK, BCL-2 antagonist/killer; BAX, BCL-2 associated X protein; BCL-2, B-cell lymphoma 2; BH3, BCL-2 homology 3; BID, BCL-2 homology domain 3 interacting-domain death agonist; Cdu1, chlamydial deubiquitinating enzyme 1; DAMPs, danger-associated molecular patterns; DD, death domain; EHEC, enterohemorrhagic *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; FADD, Fas-associated death domain; IAP, inhibitor of apoptosis protein; MCL-1, myeloid cell leukemia sequence-1; MLKL, mixed lineage kinase domain like pseudokinase; NleB1, non-Lee-encoded effector B1; PAMP, pathogen-associated molecular patterns; PARP, poly (ADP-ribose) polymerase; SidF, substrate of icm-dot transporter F; SMAC, second mitochondria-derived activator of caspases; TRADD, TNFR-associated death domain; TRAF2, TNF-receptor associated factor 2

**TABLE 1** Regulation of major cell death pathways

Cell death pathways	Apoptosis		Pyroptosis	Necroptosis
	Extrinsic	Intrinsic		
Initiated	Cell death receptors	BH3-only proteins	NLRP3, NLRC4, Aim 2, NLRP1	Cell death receptor
Regulated/mediated	Caspase-8	BCL-2 proteins, mitochondria	ASC, caspase-1, caspase-11	RIPK1, RIPK3, caspase-8
Executed	Caspase-3, caspase-7, DNFA5	Caspase-3, caspase-7, DNFA5	GSDMD	MLKL
Plasma membrane	Blebbing secondary necrosis (DNFA5)	Blebbing secondary necrosis (DNFA5)	Pore formation	Pore formation
Immune response	Silent/inflammasome activation	Silent/inflammatory over time	Inflammation (IL-1 $\beta$ , IL-18)	Inflammation (cytosolic DAMPs)

DAMP, danger-associated molecular pattern; GSDMD, gasdermin D; MLKL, mixed-lineage kinase domain like pseudokinase; DNFA5, deafness associated tumor suppressor 5.

follicular lymphoma cells and is now known to be amplified in ~90% of follicular lymphomas. The upregulation of BCL-2 directly enables continued cell proliferation and tumor growth by blocking mitochondria-mediated apoptosis (see below). Targeting BCL-2 with small molecule inhibitors, termed BCL-2 homology 3 (BH3)-mimetics, induces apoptotic cell death in cancers.<sup>15,16</sup> The BCL-2-specific BH3-mimetic ABT-199, also known as venetoclax, is now approved for the treatment of refractory chronic lymphocytic leukemia.<sup>17,18</sup>

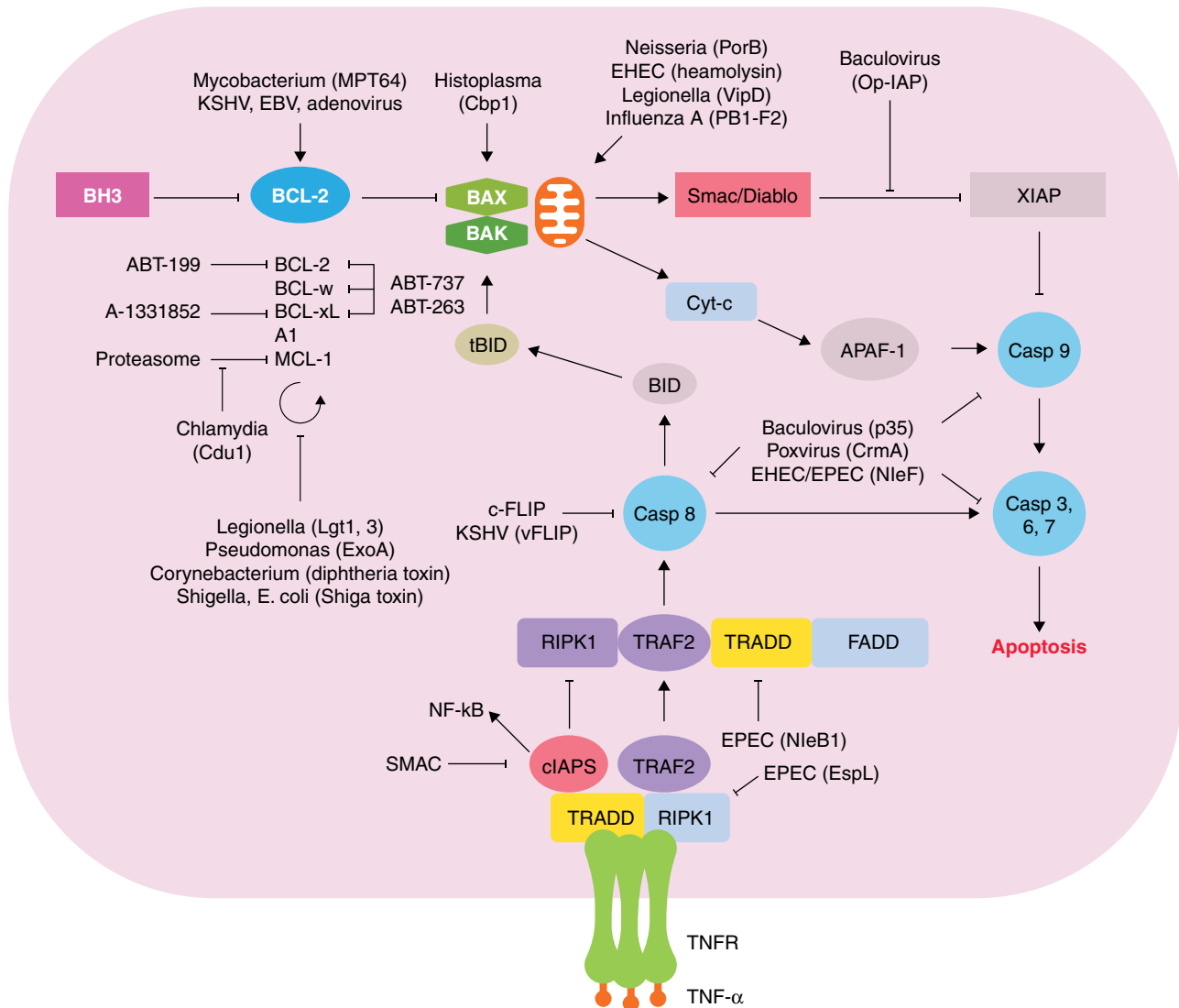
Apoptosis is also critical to maintain a functional immune system by eliminating useless or dangerous cells that recognize self-tissue. Overexpression of BCL-2 causes autoimmunity and inhibition of BCL-2 with BH3-mimetics reduces the number of selective lymphocytes.<sup>12-14,19,20</sup> Overexpression of BCL-2 also prolongs neutrophil survival during infections resulting in increased inflammation.<sup>21</sup> Conversely, the BCL-2 related protein, myeloid cell leukemia sequence-1 (MCL-1), but not BCL-2 itself, prevents the death of regulatory T-cells, which suppress inflammatory immune responses that can lead to fatal autoimmunity.<sup>22</sup> Besides maintaining homeostasis of the immune system, apoptosis may elicit immune reactions to combat invading pathogens. For instance, apoptotic bodies derived from infected cells can contain microbial products, such as pathogen-associated molecular patterns (PAMPs), that trigger protective inflammatory responses.<sup>23</sup> Furthermore, apoptotic cells themselves can activate inflammatory responses due to the release of danger-associated molecular patterns, such as mitochondrial DNA that is recognized by cytosolic receptors.<sup>24,25</sup> Apoptotic factors also regulate transcriptional programs induced by cell surface receptors to promote inflammation.<sup>7</sup> Insufficient clearance of apoptotic bodies can trigger programmed necrosis that may result in inflammation.<sup>26,27</sup> Apoptosis also orchestrates adaptive immunity as the uptake of apoptotic bodies enhances the crosspresentation of bacterial and viral antigens in dendritic cells and thus promotes protective T-cell immunity.<sup>28,29</sup> In the absence of adaptive immune responses, apoptotic cell death can directly lead to the elimination of infected cells and their pathogens.<sup>30</sup> Apoptosis also prevents the replication of intracellular pathogens and the dissemination to other organs and tissues.<sup>31</sup> While infected cells may altruistically induce cell death for the good of the host, cytotoxic lymphocytes and natural killer cells seek out infected cells and deliver the pore-forming protein, perforin, and granzymes to induce apoptosis.<sup>32</sup>

Not surprisingly, pathogens have evolved strategies to target apoptotic factors and thereby promote infections. This includes direct inhibition of cell death factors or the upregulation of host survival proteins. Recent studies demonstrate that targeting host factors with apoptosis-inducing compounds can be beneficial in infectious diseases. Before discussing the specific roles of apoptosis in infections, we will first introduce the major apoptotic factors that control the fate of host cells.

## 2 | MITOCHONDRIA-MEDIATED APOPTOSIS

Apoptosis is induced after mitochondria have been sufficiently damaged to release proteins that exclusively reside within the intermembrane mitochondrial space. The BCL-2 family members are sentinels of the mitochondria-mediated apoptosis pathway (Figure 1).<sup>33</sup> BCL-2 antagonist/killer (BAK) resides in the outer mitochondrial membrane, whereas BCL-2 associated X protein (BAX) is typically found within the cytosol of healthy cells. Activation of BAX and BAK causes their oligomerization within the outer membrane of mitochondria and subsequent membrane permeabilization.<sup>34</sup> How active BAX and BAK permeabilize membranes remains controversial but it involves conformational changes, which enable protein complex formation and membrane interactions.<sup>35</sup> BAX/BAK oligomerization and/or the formation of a membrane pore compromise mitochondrial health.<sup>36,37</sup> The activities of BAX and BAK are controlled by pro-survival BCL-2 family members, which include BCL-2, BCL-XL, BCL-W, MCL-1, and A1. The pro-survival BCL-2 members directly bind BAX and BAK and prevent their mitochondrial targeting, oligomerization, and activation.<sup>1,38</sup>

Cellular stress, as caused by infections, triggers the transcriptional upregulation of the BH3-only apoptotic factors, such as BAD, BIM, PUMA, BID (BCL-2 homology domain 3 interacting-domain death agonist), NOXA, BIK, HRK, and BMEF.<sup>39</sup> BH3-only proteins may directly activate BAX and BAK which is prevented by pro-survival BCL-2 members.<sup>40</sup> In addition, BH3-only proteins can cause the release of BAX and BAK from pro-survival BCL-2 proteins. In either case, amphipathic helices of BH3-only proteins, BAX and BAK, chiefly mediate interactions with pro-survival BCL-2 via a hydrophobic groove.<sup>39</sup> Differences in the hydrophobic groove relate to the observed



**FIGURE 1** Targeting apoptosis pathways by bacteria, viruses, and pharmacological compounds. Infections induce the mitochondria (intrinsic) or cell death receptor (extrinsic) mediated apoptosis pathway. The upregulation of BH3-only proteins (BH3) prevents pro-survival BCL-2 family members to inhibit BAX and BAK. BH3-only proteins may also be required to directly activate BAX and BAK. Active BAX and BAK cause mitochondria outer membrane permeabilization and the release of cytochrome c and SMAC/DIABLO into the cytosol. Released cytochrome c binds to apoptotic protease activating factor 1 (APAF-1), which induces the formation of the apoptosome (not shown), leading to the activation of caspases-9 (initiator caspase) and consequently caspase-3, -6, and -7 (executioner caspases). SMAC/DIABLO blocks the caspase inhibitor XIAP that regulates the caspase cascade. Extrinsic apoptosis is initiated by the oligomerization of death receptors, such as TNFR, after ligand binding, which leads to the interaction with TRADD, TRAF2, RIPK1, and cellular inhibitor of apoptosis protein (cIAPs). Recruitment of caspase-8 into a RIPK1, TRAF2, FADD, and TRADD containing cytosolic complex triggers caspase activation. Active caspase-8 cleaves caspases-3 to converge with the intrinsic pathway. In some cells and to amplify the apoptotic response, caspase-8 mediates the proteolysis of the BH3-only protein, BID, to truncated BID (tBID) that activates BAX- and BAK-mediated apoptosis. Caspase-8 also prevents RIPK3-mediated necroptosis as inhibition of cIAPs triggers caspase-8-mediated apoptosis or RIPK3-mediated necroptosis in the absence of caspase-8. In the absence of apoptosis, death receptors signal via NF- $\kappa$ B. Pathogens (and their effectors) either directly interfere with apoptosis by triggering the upregulation of BCL-2 proteins, targeting mitochondria, caspases, or death receptor signaling. Several pathogens inhibit host protein translation that primarily affects steady-state levels of MCL-1 as it is rapidly degraded by the proteasome. BH3- and SMAC-mimetic compounds inhibit pro-survival BCL-2 family members and IAPs, respectively

different interactions between pro-survival BCL-2 members with BH3-only proteins, BAX and BAK, and explain the specificity of these proteins to induce or prevent apoptosis under certain conditions. The specific interactions between BCL-2 proteins have enabled the development of small molecule inhibitors, which mimic the BH3-helix and selectively antagonize pro-survival BCL-2 members. For instance, the

BH3-mimetic ABT-737 inhibits BCL-2, BCL-XL, and BCL-W, but not MCL-1 and A1, whereas ABT-199 only blocks the activity of BCL-2.<sup>18</sup> In so doing, BH3-mimetic compounds unleash BAX and BAK from the pro-survival BCL-2 members.

Activation of BAX and BAK is considered to be the step of no return, which ultimately leads to cell death. This is partly explained

by BAX- and BAK-mediated release of cytochrome *c* into the cytosol, as cytochrome *c* is required to maintain the mitochondrial respiratory chain and mitochondrial membrane potential and to scavenge reactive oxygen species.<sup>41,42</sup> Furthermore, released cytochrome *c* activates a signaling cascade that triggers the activation of asparagine-specific cysteine proteases, known as caspases.<sup>43,44</sup> Another released mitochondrial protein, termed second mitochondria-derived activator of caspases (SMAC) (also known as DIABLO), antagonizes the cytosolic caspase X-linked inhibitor of apoptosis protein (XIAP), and thus further promotes the activity of caspase-9.<sup>45,46</sup> Active caspase-9 cleaves executioner caspases, such as caspase-3, -6, and -7, to fast track the dismantling of the cell by activating DNases and inactivating DNA repair proteins, such as poly (ADP-ribose) polymerase.<sup>47,48</sup>

There are at least 19 members of the human BCL-2 family.<sup>1</sup> Loss of individual BH3-only proteins typically results in mild abnormal phenotypes in mice, suggesting redundancy between the different death factors.<sup>39</sup> So far, only a limited number of studies have reported the use of knockout mice to determine the role of BH3-only proteins in preclinical infection models.<sup>49</sup> In contrast, the BCL-2 pro-survival factors are essential for development resulting in death before or shortly after birth.<sup>39</sup> In the absence of knockout mice, studies to determine the role of BCL-2 family members in infections have been largely limited to cultured cell lines. Mice overexpressing BCL-2 have also been used to determine the role of mitochondria-mediated cell death suggesting that apoptosis promotes pathogen clearance and/or dampens immune responses in infections.<sup>21,50</sup>

### 3 | CELL DEATH RECEPTOR-MEDIATED APOPTOSIS

Apoptosis can also be induced by extracellular signals that activate cell death receptors (Figure 1). These trans-membrane receptors belong to the TNF superfamily comprising 29 receptors and 19 ligands.<sup>39</sup> TNF signaling regulates cell survival, proliferation, differentiation, and inflammation, but also apoptosis.<sup>51</sup> The TNF glycoprotein was initially identified as an endotoxin-induced protein capable of causing tumor necrosis over 40 years ago.<sup>52</sup> We now know that TNF- $\alpha$ , also known as cachectin, is produced by many cell types to induce inflammation and cell death after sensing invading pathogens.<sup>53,54</sup> Binding of TNF- $\alpha$  to its cell surface receptor, TNFR1, triggers the recruitment of TNFR-associated death domain (TRADD) protein through its death domain (DD), as well as the receptor-interacting protein (RIPK1), TNF-receptor associated factor 2 (TRAF2), and cellular inhibitor of apoptosis protein (cIAP).<sup>55</sup> TRADD recruits procaspase-8 to form the death-inducing signaling complex or a RIPK1/TRAF2/TRADD/FADD/caspase-8 complex (Figure 1). Both cause autoactivation of caspase-8 that cleaves caspase-3 to induce apoptosis.<sup>8</sup> In some cell types, caspase-8 may also cleave the BH3-only protein, BID. The truncated BID form in turn activates BAX and BAK to induce mitochondria-mediated apoptosis, which includes the release of cytochrome *c* and activation of downstream apoptotic caspases.<sup>56</sup>

Of note, recent studies have highlighted that caspase-8 can modulate the activities of other cell death pathways. Depending on the stimulus and cell type, caspase-8 regulates the activity of the nuclear factor- $\kappa$ B (NF- $\kappa$ B) transcription factor family and thus the expression of inflammatory cytokines and death factors such as IL-1 $\beta$  and NLRP3, respectively.<sup>57</sup> Caspase-8 may also posttranscriptionally regulate the NLRP3 inflammasome, which can involve direct cleavage of IL-1 $\beta$  and caspase-1.<sup>58–60</sup> In the absence of caspase-1, caspase-8 may trigger inflammasome-dependent cell death.<sup>59,61–63</sup> Caspase-8 is also known to prevent a caspase-independent form of cell death, which is mediated through RIPK1, RIPK3, and mixed lineage kinase domain like pseudokinase (MLKL) and leads to necroptosis.<sup>64</sup> These recent findings are likely to lead to many more examples of crosstalk between the different cell death pathways. As pathogens can subvert apoptosis, activating alternative programmed cell death pathways is thought of as a backup mechanism that enables compromised cells to alert other immune cells of a persisting and thus potentially dangerous pathogen.<sup>7</sup>

### 4 | PATHOGEN-MEDIATED INHIBITION OF APOPTOSIS

PAMPs and death ligands that are secreted due to infections can activate extrinsic apoptosis. Infections also induce intrinsic apoptosis due to DNA damage, metabolic stress, and calcium signaling. Signaling pathways that converge on the NF- $\kappa$ B transcription factor family upregulate the expression of apoptotic survival and death factors. Thus, successful pathogens have evolved several mechanisms to subvert apoptosis as highlighted by selected examples (see Table 2).

*Legionella pneumophila* primarily replicates in lung macrophages during infections by blocking apoptosis. The bacterial effector protein substrate of icm-dot transporter F (SidF) is thought to inhibit BH3-only proteins, which are transcriptionally upregulated in a NF- $\kappa$ B-dependent manner in *Legionella*-infected macrophages.<sup>65–67</sup> Loss of SidF, however, does not necessarily lead to increased macrophage death in *Legionella* infections.<sup>68</sup> Furthermore, bacterial replication and macrophage cell death are not affected by the genetic deletion of several BH3-only proteins.<sup>68,69</sup> Apoptosis may thus be blocked by the transcriptional upregulation of pro-survival BCL-2 family members, including BCL-2 and A1 in a NF- $\kappa$ B-dependent manner.<sup>65,66</sup> The protein levels of pro-survival BCL-2 members, however, do not increase in infected macrophages, in agreement with the observation that *Legionella* inhibits host protein translation.<sup>69</sup> While the inhibition of BCL-2 with the BH3-mimetic ABT-199 fails to activate apoptosis, loss of the related protein BCL-XL induces cell death of *Legionella*-infected macrophages and prevents bacterial growth.<sup>69</sup> The unique role of BCL-XL to prevent apoptosis in infections is dependent on MCL-1.<sup>69</sup> Unlike other pro-survival factors, MCL-1 is highly unstable with a half-life of ~30 min.<sup>70</sup> Inhibition of host protein translation by the *L. pneumophila* effectors, Lgt1 and Lgt3, is sufficient to trigger the rapid loss of MCL-1.<sup>69</sup> Under these conditions, BCL-XL is the sole pro-survival BCL-2 family member able to effectively prevent the activation of BAX and BAK to induce apoptosis.

**TABLE 2** Examples of how pathogens activate or inhibit apoptosis

Pathogen	Effector	Host target	Apoptosis	Reference
<i>Legionella pneumophila</i>	SidF	BNIP3, BCL-RAMBO	Inhibition/no effect	67,68
	Lgt 1	Elongation factor 1A	Activation	69
	Lgt 3	Elongation factor 1A	Activation	69
	VipD	Phosphatidylcholine, phosphatidylethanolamine	Activation	120
	LegS2	Sphingolipids	Activation	120
	NcP	Mitochondrial nucleotides	unknown	121
<i>Chlamydia trachomatis</i>	CPAF/unknown	BH3-only proteins (BAD)	Inhibition	71-74
	Cdu1	MCL-1	Inhibition	77
<i>Mycobacterium tuberculosis</i>	MPT64	miRNA-21 and BCL-2	Inhibition	78
<i>Neisseria meningitidis</i>	PorB	Mitochondria	Inhibition	103
<i>Neisseria gonorrhoeae</i>	PorB	Mitochondria	Activation	101
<i>Corynebacterium diphtheriae</i>	Diphtheria toxin	Elongation factor 2	Activation	125
<i>Pseudomonas aeruginosa</i>	Exotoxin A	Elongation factor 2	Activation	69
<i>Escherichia coli</i>	Hemolysin	Mitochondria	Activation	99,100
	NleF	Caspase-8, -9, and -3	Inhibition	93-95
	NleB1	FADD, TRADD, TNFR1	Inhibition	96,97
<i>Histoplasma capsulatum</i>	Cbp1	unknown	Activation	117
$\gamma$ -Herpesviruses	vFLIP	Caspase-8	Inhibition	90-92
Influenza A	PB1-F2	Mitochondria	Activation	115
<i>Baculoviruses</i>	Op-IAP	Caspase-3, -7, and -9	Inhibition	89
	p35	Caspases	Inhibition	92
<i>Poxviruses</i>	CrmA	Caspase-8 and -10	Inhibition	92

Other pathogens target key apoptotic factors to prevent apoptosis (Figure 1). *Chlamydia trachomatis* translocates a serine protease into the cytosol of infected macrophages to degrade BH3-only factors, such as BAD.<sup>71-73</sup> *Chlamydia* infections also trigger the phosphorylation of BAD and its sequestration to the bacteria containing vacuole to prevent mitochondria targeting and apoptosis.<sup>74</sup> In addition, *Chlamydia* induces the expression of pro-survival MCL-1 and genetic deletion of MCL-1 sensitizes infected cells to apoptosis.<sup>75,76</sup> Given that MCL-1 is rapidly degraded via the ubiquitin-proteasome degradation pathways, *Chlamydia* must also employ other mechanisms to prevent MCL-1 loss. This includes the chlamydial deubiquitination enzyme 1 (Cdu1), which targets ubiquitinated MCL-1 and prevents its proteasomal degradation.<sup>77</sup> Thus, Cdu1 promotes *Chlamydia* infections by preventing apoptosis of infected cells, although other host targets and roles remain to be identified.<sup>77</sup>

Many other bacterial pathogens induce the expression of pro-survival BCL-2 family members by activating the NK- $\kappa$ B signaling pathways. In general, this prevents apoptosis and promotes intracellular replication, as is the case with *Mycobacterium tuberculosis*. Secretion of *M. tuberculosis* protein, MPT64, modulates the expression of host miRNA-21, which in turn increased NF- $\kappa$ B signaling and BCL-2 expression.<sup>78</sup> In addition, MCL-1 is reported to be upregulated in *M. tuberculosis* infected macrophages and knockdown of MCL-1 triggers apoptosis and prevents bacterial replication.<sup>79</sup> In contrast, several viruses, including adenovirus, Kaposi's sarcoma associated

herpesvirus, Epstein-Barr virus, and murine  $\gamma$ -herpesvirus 68, encode BCL-2 like proteins that share structural similarities with mammalian BCL-2 and inhibit BAX/BAK-mediated apoptosis.<sup>80-82</sup> The BCL-2 ortholog of  $\gamma$ -herpesvirus promotes the survival of infected immature B-cells, which would otherwise be eliminated by negative selection and apoptosis, and enables life-long viral infection.<sup>83</sup> Besides targeting mitochondria and inhibiting apoptosis, these viral BCL-2 orthologs may also fulfill additional roles, as replacement with mammalian pro-survival BCL-2 members is insufficient to rescue viral replication.<sup>84</sup> Other viruses also inhibit BAX and BAK activation even though they encode proteins that show very little similarities to mammalian BCL-2 family members, as is the case in poxviruses.<sup>85-87</sup> This suggests that inhibition of BAX/BAK-mediated apoptosis is important to establish viral infections and to promote efficient viral replication.<sup>88</sup>

Besides blocking BAX and BAK activation, several viruses inhibit apoptotic caspases by molecular mimicry. For instance, baculoviruses encode IAP homologs to inhibit host caspases and apoptosis.<sup>89</sup> Kaposi's sarcoma associated herpesvirus impairs BAX and BAK but also caspase-8 activation. The latter depends on the expression of the viral FADD-like ICE (FLICE) inhibitory protein, which mimics human cFLIP by acting as a dominant-inhibitory molecule.<sup>90,91</sup> In addition, viruses may express pan-caspase inhibitors that do not show obvious similarities to known human genes to suppress a range of caspases.<sup>92</sup> It is possible that these provide an additional safeguard in



preventing BAX- and BAK-mediated apoptosis. Recent observations also suggest that activating BAX and BAK in the absence of caspases triggers antiviral immune responses due to the release of mitochondrial DNA. Cytosolic DNA is sensed by cyclic GMP-AMP synthase and induces inflammatory responses.<sup>24,25</sup> Thus, inhibiting BAX and BAK, as well as caspases, may have been an evolutionary pressure to not only prevent host cell death but also inflammatory responses known to be highly effective to eliminate viral pathogens.

Bacterial pathogens, too, target host caspases to block their activity. Enteropathogenic and enterohemorrhagic *Escherichia coli* (EPEC/EHEC) secrete the effector, NleF, which prevents the activation of apoptotic caspases with similar potency as endogenous IAPs.<sup>93-95</sup> In addition, the EPEC effector non-Lee-encoded effector B1 (NleB1) interacts with the DD of Fas-associated death domain (FADD), TRADD, TNFR1, and RIPK1 and specifically inhibits extrinsic apoptosis.<sup>96,97</sup> NleB1 possess glycosyltransferase activity and modifies the DD with a single N-acetyl-D-glucosamine (GlcNAc) residue at a conserved arginine residue.<sup>96,97</sup> This highly unusual glycosylation is irreversible, as it is not recognized by host glucosidases, preventing the formation of the death-inducing signaling complex and hence caspase-8 activity. Activation of apoptosis is critical to clear gut pathogens by shedding infected epithelial cells. Consistent with this, EPEC-like *Citrobacter rodentium* lacking NleB fails to establish infections in wild-type mice, but not in mice deficient in extrinsic apoptosis.<sup>97</sup> Given that inhibition of caspase-8 may trigger activation of RIPK1-, RIPK3-, and MLKL-mediated necroptosis, EPEC relies on another effector, EPEC-secreted/signaling protein L (EspL), to cleave RIPK1 and RIPK3 to prevent necroptosis.<sup>98</sup>

These studies highlight the different strategies employed by pathogens to inhibit host cell apoptosis. Discovering these strategies has enabled the identification of new host cell death pathways and their roles in preventing infectious diseases.

## 5 | ACTIVATION OF APOPTOSIS BY PATHOGENS

Paradoxically, pathogens that inhibit apoptosis may also target mitochondria to induce apoptotic cell death (Table 2). Apoptosis can promote bacterial and viral infections by eliminating immune or epithelial cells that act as initial barrier in infections. Apoptosis, rather than other forms of programmed cell death, is thought to be the preferred pathway under these conditions, as it generally does not elicit inflammatory responses. Consistent with this, EHEC-secreted hemolysin targets mitochondria of epithelial cells to trigger cytochrome *c* release and the activation of apoptotic caspases.<sup>99,100</sup> The porin PorB from pathogenic *Neisseria* targets the mitochondria by utilizing the host import machinery.<sup>101,102</sup> However, not all porins behave similar, as *N. meningitidis* PorB prevents apoptosis, whereas PorB from *N. gonorrhoeae* sensitizes cells to apoptosis, suggesting they have different functions in infections.<sup>103,104</sup> PorB-induced apoptosis is thought to be mediated by directly compromising the mitochondrial membrane integrity due to pore formation of the toxin, but also by activating BH3-

only factors, such as BIM and BMF, to ultimately induce the demise of the cell in a BAX- and BAK-dependent manner.<sup>105,106</sup> PorB-induced apoptosis is thought to promote infection by disrupting the epithelial barrier, although this awaits further experimental validation that has been hampered by the inability to genetically manipulate PorB in *N. gonorrhoeae*.

In viral infections, activation of apoptosis can also be detrimental to the host by promoting viral dissemination and immune cell death. Human immunodeficiency virus (HIV) can activate apoptosis in infected activated T-cells, although it induces pyroptosis in nonproductive infections of resting T-cells.<sup>107-109</sup> Other viral infections trigger Fas-mediated apoptosis as observed in reovirus-infected mice that show apoptotic neuronal cell death and encephalitis.<sup>110</sup> Similarly, chronic murine  $\gamma$ -herpesvirus infection triggers the loss of T-cells depending on BIM- and Fas-mediated apoptosis.<sup>111</sup> The upregulation of Fas and its ligand FasL also regulates the life-span of important immune cells, including neutrophils and macrophages, during viral infections.<sup>112</sup> Pro-survival factors can act to prevent Fas-mediated apoptosis as evidenced by the increased expression of MCL-1 due to TLR signaling within an inflammatory milieu, which causes detrimental inflammatory complications as observed in influenza infections.<sup>113</sup> Conversely, accelerated apoptosis of neutrophils causes neutropenia, which is correlated with increased susceptibility to several infections.<sup>113</sup> Based on recent studies, viral proteins can directly target mitochondria to induce apoptosis in immune cells. For instance, the influenza A virus protein polymerase basic protein 1-frame 2 (PB1-F2) targets mitochondria resulting in loss of mitochondrial membrane potential and apoptosis.<sup>114</sup> PB1-F2 preferentially induces apoptosis in alveolar macrophages and thus promotes infections as macrophages are critical to orchestrate immunity during the early stages of infections. The activity of PB1-F2 is blocked by the NOD-like receptor family member, NLRX1, which prevents influenza-mediated apoptosis in macrophages.<sup>115</sup> NLRX1 is reported to maintain the integrity of mitochondria and to prevent macrophage apoptosis and inflammatory signaling that may be damaging in influenza infections.<sup>115</sup>

Intracellular microbial pathogens also induce apoptotic death of host cells after successful replication to aid in escape and reinfection of new cells. For instance, apoptosis of *Leishmania*-infected macrophages triggers the phagocytosis of parasites within apoptotic bodies by uninfected macrophages to perpetuate infection.<sup>116</sup> The fungal pathogen *Histoplasma capsulatum* secretes calcium-binding protein 1 (Cbp1) to trigger BAX- and BAK-mediated caspase activation, which is sufficiently lytic to promote escape from macrophages.<sup>117,118</sup> Overexpression of BCL-2 also reduces macrophage death in late-stage *Legionella* infections,<sup>21,119</sup> suggesting that *Legionella* activates apoptosis to escape from spent cells. Other cell death pathways likely contribute to *Legionella*-mediated killing of macrophages as well.<sup>68</sup> In contrast to permissive macrophages, dendritic cells rapidly induce apoptosis after *Legionella* infection, partly because several bacterial effectors compromise mitochondria health.<sup>120-122</sup> This suggests that some pathogens can sense their host cell to trigger apoptosis, although the molecular mechanism behind this remains unclear. It is possible that dendritic cells express additional factors that promote the

activity of mitochondria-targeted *Legionella* effectors.<sup>120</sup> While the overexpression of BCL-2 prevents apoptosis of *Legionella*-infected dendritic cells and enables bacterial replication,<sup>31</sup> the effectors induce apoptosis independent of BAX and BAK, suggesting that *Legionella* activates multiple cell death pathways.<sup>120</sup>

Besides targeting mitochondria to induce apoptosis, several pathogens inhibit host protein translation to disarm immune cells. Arresting protein translation is a well-established inducer of mitochondria-mediated apoptosis depending on BAX and BAK activation.<sup>40,123</sup> Inhibiting protein translation potentially generates a cellular stress response, which may lead to the posttranslational activation of BH3-only factors. In addition, arresting protein translation causes the loss of important pro-survival factors, such as MCL-1, which may be sufficient to trigger BAX/BAK-mediated apoptosis under these conditions.<sup>124</sup> Consistent with this view, translation inhibitors cause delayed cell death that follows a gradual loss of cell viability in a caspase-dependent manner.<sup>68</sup> It is conceivable that intracellular pathogens efficiently replicate under these conditions. While chemical inhibition and pathogen-mediated blockade of protein translation share several phenotypes (loss of mitochondrial potential, cell death kinetics), the latter may not necessarily lead to the activation of caspase-3/7 activity, as observed in *Legionella* infections.<sup>68</sup> A lack of apoptotic caspase activity is consistent with recent observations that *Legionella* growth and macrophage killing proceeded normally in the absence of caspase-3 and BAX/BAK or in the presence of caspase inhibitors.<sup>31,68,69</sup> Whether *Legionella* actively inhibits the activity of apoptotic caspases, as observed in viral and other bacterial infections, remains unknown.

Extracellular pathogens also target ribosomes to arrest host protein translation and to induce apoptosis.<sup>125</sup> *Corynebacterium diphtheriae* and *Pseudomonas aeruginosa* secrete diphtheria toxin and exotoxin A, respectively.<sup>125</sup> Both toxins catalyze the transfer of adenosine diphosphate (ADP) ribose to elongation factor 2, which inhibits translation elongation. As observed in *Legionella* infections, exotoxin A also caused the rapid depletion of MCL-1 in macrophages and delayed cell death associated with caspase-3 cleavage.<sup>69</sup> Cell death under these conditions is accelerated in the absence of BCL-XL.<sup>69</sup> These exotoxins are major virulence factors but their specific roles in infections remains unclear. It is possible that inducing apoptosis in macrophages may enable immune evasion by extracellular pathogens. It should be noted, however, that the translational arrest induced by diphtheria toxin, exotoxin A, and *Legionella* effectors triggers the secretion of inflammatory cytokines.<sup>126–129</sup> These cytokines are preferentially translated by ribosomes under attack by pathogens due to high-copy mRNA numbers induced by Toll-like receptor signaling, although alternative mechanisms may be at play that ensures their translation.<sup>127,128,130</sup> Furthermore, exotoxin A may also trigger pyroptosis as exotoxin-deficient *Pseudomonas* mutants trigger reduced levels of pyroptotic death and cytokine secretion in macrophages.<sup>131</sup> This suggests an evolutionary arms race whereby bacteria have evolved virulence factors to disarm important defense cells and whereby wounded immune cells have evolved mechanism to alert other cells of a potentially dangerous intruder.

## 6 | TARGETING APOPTOTIC FACTORS TO TREAT INFECTIONS

Several pathogens trigger the upregulation of BCL-2 family members to prevent apoptosis and to perpetuate infections. This raises the possibility that targeting BCL-2 family members to induce apoptosis could be exploited as an alternative strategy to combat infections. Indeed, targeting BCL-XL with BH3-mimetic compounds, originally developed to induce cancer cell death, activates apoptosis in *Legionella*-infected macrophages. This is sufficient to prevent bacterial replication in infected lungs.<sup>69</sup> Because uninfected macrophages and other cells maintain MCL-1 expression, they remain protected from BCL-XL-inhibiting compounds.<sup>69</sup> As such, BH3-mimetic treatment of *Legionella*-infected mice only causes death of infected cells without compromising the immune system. The rapid induction of apoptosis by BH3-mimetic administration also reduces excessive inflammation, likely by preventing the secretion of pro-inflammatory cytokines in infected macrophages.<sup>69</sup> It is possible that both, controlling inflammation and clearing *Legionella*, maintain animal health despite life-threatening infections.

Viruses also manipulate host protein translation, suggesting that similar strategies may be applicable more widely in infectious diseases. In support of this, vaccinia virus and cytomegalovirus infections of macrophages and epithelial cells cause the depletion of MCL-1 and, consequently, loss of BCL-XL induces rapid apoptosis.<sup>132</sup> As observed in *Legionella* infections, pharmacological inhibition of BCL-XL with the BH3-mimetic compound ABT-737 reduces viral production in vitro.<sup>132</sup> Whether this leads to increased survival in preclinical infection models and is applicable to other viral infections has not been determined so far. Similarly, targeting BCL-2 with the BH3-mimetic compound ABT-199 induces apoptosis of T-cells harboring latent but also productive HIV infections.<sup>133</sup> This reduces the HIV reservoir and the spreading of infections in ex vivo models. Inducing apoptosis also decreases hepatitis B viral loads in preclinical infection models.<sup>134,135</sup> The SMAC-mimetic compound birinapant causes the degradation of endogenous IAPs and triggers TNF- $\alpha$ -mediated extrinsic apoptosis.<sup>136</sup> While TNF- $\alpha$  is critical to control hepatitis B infections in mice and humans by promoting inflammatory responses, inducing apoptosis via SMAC-mimetic administration is effective in clearing chronic viral infections.<sup>134</sup>

## 7 | CONCLUDING REMARKS

Over the last three decades, the roles of individual apoptotic factors have been elucidated using transgenic animal model systems. The development of BH3-mimetic compounds that specifically target BCL-2 family members have further advanced our understanding of how these proteins control development and immunity, but also promote cancer. The BH3-mimetic ABT-263 (navitoclax) is effective against chronic lymphocytic leukemia, but also causes thrombocytopenia in early phase clinical trials. Loss of platelets is due to the inhibition of BCL-XL rather BCL-2.<sup>137</sup> Consequently, the development of ABT-199 (venetoclax) as a BCL-2-specific BH3-mimetic compound resulted in

the approval by the Food and Drug Administration for the treatment of refractory chronic lymphocytic leukemia. Additional BH3-mimetic compounds including those that specifically inhibit BCL-XL and MCL-1 are under further development for the treatment of cancers that remain refractory to ABT-199.<sup>138,139</sup>

Whether similar strategies will be beneficial in infectious diseases is only just being investigated. The use of transgenic animal models has now identified BCL-XL as the Achilles heel in *Legionella* and viral infections. BCL-XL-specific BH3-mimetic compounds as well as ABT-263 proved beneficial in preclinical animal models of *Legionella* infections. Whether low doses and/or single administration of ABT-263 will be sufficient in humans to eliminate bacterial and viral infections without causing thrombocytopenia remains to be determined. Inducing apoptosis with the SMAC-mimetic compound birinapant reduces chronic hepatitis B infections in mice. As with venetoclax, birinapant was initially developed to kill cancer cells by inducing apoptosis. Birinapant has a good safety profile in humans and is in early phase clinical trials in combination with current antiviral therapies. This highlights the possibility of repurposing apoptosis-inducing drugs to treat infectious diseases with the economic benefit of accelerating the path from bench to bedside. The different mechanisms employed by pathogens to subvert host apoptotic pathways, however, will necessitate further proof-of-concept investments to deliver clinically meaningful outcomes.

## AUTHORSHIP

M.C.F. and T.N. wrote the manuscript and prepared the figure.

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## DISCLOSURES

The authors declare no conflict of interest.

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