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Regulation of Apoptosis by Gram-Positive Bacteria:

Mechanistic Diversity and Consequences for Immunity

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Abstract

Apoptosis, or programmed cell death (PCD), is an important physiological mechanism, through which the human immune system regulates homeostasis and responds to diverse forms of cellular damage. PCD may also be involved in immune counteraction to microbial infection. Over the past decade, the amount of research on bacteria-induced PCD has grown tremendously, and the implications of this mechanism on immunity are being elucidated. Some pathogenic bacteria actively trigger the suicide response in critical lineages of leukocytes that orchestrate both the innate and adaptive immune responses; other bacteria proactively prevent PCD to benefit their own survival and persistence. Currently, the microbial virulence factors, which represent the keys to unlocking the suicide response in host cells, are a primary focus of this field. In this review, we discuss these bacterial "apoptosis regulatory molecules" and the apoptotic events they either trigger or prevent, the host target cells of this regulatory activity, and the possible ramifications for immunity to infection. Gram-positive pathogens including Staphylococcus, Streptococcus, Bacillus, Listeria, and Clostridia species are discussed as important agents of human infection that modulate PCD pathways in eukaryotic cells.

Keywords

Bacteria; apoptosis; programmed cell death; pathogenesis; immunity

INTRODUCTION

A number of excellent reviews have been published in recent years on the topic of bacteria-induced apoptosis, or programmed cell death (PCD) [1-7]. These comprehensive reports have brought together the plethora of research studies conducted over the past decade in this field, beginning with the first demonstration that enteropathogenic *Shigella* induces PCD in macrophages under certain conditions [8]. Following that seminal study by Zychlinsky *et al*. (1992), other investigators illustrated convincingly that many other species of pathogenic bacteria such as *Streptococcus* [9] and *Mycobacterium* [10] and certain viruses, including human immunodeficiency virus (HIV) [11], directly manipulate the apoptotic machinery within human cells to benefit their own survival and persistence. This explosion of new information on bacteria-induced apoptosis and the diverse molecular mechanisms triggered by bacteria to bring about PCD argues that apoptosis plays a key role in microbial pathogenesis and antibacterial immunity.

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Gram-negative and gram-positive bacteria can trigger the suicide response in selected lineages of eukaryotic host cells [12,13]. This suggests that some common bacterial structural component may function meaningfully or coincidentally with infection to bring about PCD in the harboring host cell. To some degree, this concept is supported by available data on genera such as *Mycobacterium*, in which purified lipoarabinomannan or its precursors from different species influences apoptotic pathways (albeit in contrasting ways) [14-16]. In larger divisions of bacteria such as gram-negative Gracilicutes [17] the lipopolysaccharide component of the outer cell membrane exerts a proapoptotic effect in several dissimilar situations [13,18,19], although it can also prevent PCD in certain cell types under different conditions [20,21]. In the case of gram-positive pathogens, there have been few reports of shared molecules that are able to modulate PCD pathways. Moreover, it appears that the molecular mechanisms of PCD induced by gram-positive bacteria differ from those triggered by their gram-negative counterparts [22]. One conserved gram-positive factor studied to date is lipotechoic acid, though investigations into its role as a PCD regulator in eukaryotic host cells have indicated disparate effects on different cell types [23-25]. Other apoptogenic molecules identified in gram-positive pathogens so far include exotoxins (e.g., lethal factor of *Bacillus anthracis*) [25,26], cytolysins (e.g., listeriolysin O of *Listeria monocytogenes*) [27], and bacterial hemolysins (e.g., β-hemolysin of *Streptococcus agalactiae*) [28,29].

At the most basic level, apoptosis regulatory molecules (ARMs) in gram-positive bacteria can be classified as secreted exoproducts (e.g., exotoxins such as pneumolysin) [30] and nonsecreted endoproducts (e.g., lipotechoic acid) [31]. Functionally, the molecular mechanisms resulting in PCD that are brought about by gram-positive bacteria are heterogeneous. PCD pathways involving both the intrinsic mechanism (i.e., mitochondrial function disruption) [32,33] and extrinsic signaling through death receptors [34,35] are triggered by various gram-positive bacteria (Table **1**). Thus, similar to PCD triggered by parasitic infections [36], there appears to be limited conservation of the mechanisms underlying the PCD response of eukaryotic host cells to gram-positive bacteria. This review summarizes the current knowledge about ARMs produced by gram-positive bacteria, their known target cell repertoire, the PCD events that these ARMs trigger, and the potential consequences for immunity to infection.

STEADY-STATE PCD MACHINERY AND FUNCTION

Apoptosis plays an important role in immune system function; PCD that occurs normally in development and homeostasis is balanced with that stimulated after cytotoxic insult, metabolic imbalance, or infectious attack [151-156]. Removal of useless or autoreactive cells by apoptotic clonal deletion (negative selection) is central to the development of B- and T-cell repertoires. In immune responses to infection, PCD maintains appropriately sized T-cell memory pools after disease resolution by PCD-driven removal of antigen-expanded T-cell clones [157-160]. PCD also regulates the balance between T-cell proliferation and T-cell death in some infections [161-163]. Human cells commit to PCD *via* one of two generalized activation pathways: First, death receptor-independent deregulation of mitochondrial function, during which cytosolic cytochrome *c* binds with apoptotic protease activating factor-1 (APAf-1) and cleaved procaspase-9 to form the apoptosome (intrinsic PCD) [32,33,164-168]. Second, activation of the death receptor pathway through ligation of CD95 (FAS/APO-1) [169], tumor necrosis factor (TNF)-α receptor 1, or other death receptors at the eukaryotic cell surface may lead to the activation of caspase-8, cleavage of procaspase-3 (or effector caspases-6 or -7), and terminal extrinsic PCD events [34,35,170] (Fig. **1**). Toxic proteins released from cytotoxic lymphocytes and natural killer (NK) cells such as perforin and granzyme B may also activate extrinsic PCD [171]. Both pathways are tightly regulated so that under normal conditions unnecessarily high levels of energy-dependent apoptosis are circumvented [172], yet a state of readiness is maintained. The capacity for rapid PCD responses, which can be completed within

30 minutes of initial signaling, is tied to the presence of large pools of enzymatically inactive forms of cysteine-dependent aspartate-directed specific proteases (caspases) within the cytoplasm of eukaryotic cells under resting conditions [34,173,174]. In human cells, a family of at least 13 caspases act in concert, functioning as initiators (e.g., caspase-8, -9, -10) and effectors (e.g., caspase-3, -6, -7) of PCD [175-179]. Caspases can also cleave proforms of cytokines, such as interleukin-1, that are involved in inflammation [176]. Procaspase zymogens undergo rapid proteolytic cleavage at specific aspartate residues, which can be self-induced or triggered by other caspases after appropriate proapoptotic stimulation [173,177]. Upon reaching a critical threshold, complete execution of PCD is achieved, resulting in cleavage of protein substrates required for cellular integrity, e.g., poly(ADP-ribose)polymerase (PARP), inhibitor of caspase-activated deoxyribonuclease (ICAD), and DNA degradation [180-182]. Caspases target as many as 280 proteins [183]. Functional redundancy and compensation in the caspase system ensures PCD can be achieved when necessary [184]. Ultimately, both PCD pathways converge at the point of effector caspase-3 or -6 activation [160]. This leads to chromatin condensation, externalization of cell membrane lipid phosphatidylserine [185], membrane blebbing, cell shrinkage, and cell disassembly into apoptotic bodies. Phagocytosis of suicidal cells and apoptotic bodies prevents the spillage of intracellular contents from dying cells, thereby limiting inflammation [173]. Macrophages and other phagocytic cells recognize apoptotic cells by phosphatidylserine receptors [186], and can actually lead to the release of anti-inflammatory cytokines [187] and desensitization [188]. Complex regulatory checkpoints for PCD exist at all levels of transcription, translation and posttranslational modification [177]. In particular, the network of nuclear factor κB (NF-κB) transcription factors lies at the crossroads of many PCD signaling pathways and typically exerts prosurvival influence [189]. An additional transcription factor that is central to the regulation of PCD is p53, which influences cell cycle arrest, DNA repair, cell proliferation, and can induce PCD in cells harboring genetic mutations [190].

Intrinsic Mitochondria-Mediated PCD

Once the intrinsic pathway of PCD is triggered, the ensuing cellular changes are characterized by loss of mitochondrial transmembrane potential ($\Delta\Psi m$) and cellular reduction-oxidation steady state, disruption of the electron transport chain, oxidative phosphorylation, and adenosine triphosphate (ATP) production, and release of proteins that activate caspases [32, 33]. Environmental cues that can induce intrinsic PCD include stressors such as cytotoxic treatments (e.g., UV light, irradiation), depletion of essential nutrients or growth factors (e.g., cytokines), chemical imbalances (e.g., Ca^{2+} overload) and chemotherapeutic insults [160, 191]. Several bacterial toxins also stimulant intrinsic PCD [163]. Cytotoxic signals open permeability transition pores in the inner mitochondrial membrane, which induces loss of membrane potential. Subsequent release of mitochondrial cytochrome *c* into the cytoplasm may occur as a result of swelling of the mitochondria and outer membrane rupture. Alternatively, direct pore formation in the outer mitochondrial membrane due to the actions of BAK and/or BAX may facilitate the passage of cytochrome *c*. Free cytochrome *c* then binds to APAf-1 in the cytoplasm [177,192,193]. Subsequent binding with procaspase-9 forms the apoptosome, which leads to cleavage and activation of caspase-3 [177] and PCD (Fig. **1**). Mitochondria-induced PCD can also occur independently of caspase activation [194]. Intrinsic PCD is regulated by an intricate system of agonistic and antagonistic mitochondrial proteins that display sequence and structural similarity to the B-cell leukemia (BCL)-2 homology domain [160,195]. Proteins containing BCL-2 homology domains (BH1 through BH4) regulate the balance of PCD events occurring at the mitochondria by homodimerizing, heterodimerizing, and neutralizing the activities of functionally competing partner proteins through complementary binding domains [191,196]. A key member of the BCL-2 family of proteins is BCL-X_L, which resides in the inner mitochondrial membrane space and promotes cell survival by binding to and suppressing the activities of apoptogenic BCL-2 family member

proteins such as BAX [191,196]. Twenty BCL-2 family member proteins have been identified: A1, BCL-W, BCL-X_L, BOO, MCL-1, BAX, BAK, BOK, BCL-X_S, BCL-G_L, BAD, BCL-G_S, BID, BIK, BIM, BLK, BMF, HRK, NOXA and PUMA (reviewed in [160]). Several gram-positive bacteria and their ARMs manipulate the levels, phosphorylation status, and functional availability of BCL-2 homology proteins, thereby influencing intrinsic PCD.

Extrinsic Death Receptor-Mediated PCD

Death receptors on the surface of host cells include CD95, TNF receptors, and death receptors 3 through 5 [34,35,170,197]. Ligation of death receptors induces cleavage of procaspase-8 (also called FLICE), which, in turn, activates procaspase-3 and leads to DNA fragmentation and cell death (Fig. **1**) [34]. Signal transduction *via* recruitment of the FAS-associated death domain (FADD) adaptor protein and formation of the death-inducing signaling complex is essential for caspase-8 activation by both CD95-FAS ligand (FASL) and the TNF-TNF receptor 1-TNF receptor-associated death domain (TRADD) adaptor protein complex [34]. Transmembrane death receptors initiate PCD by binding to death domain regions of cytoplasmic adaptor proteins, causing structural rearrangements and recruitment of procaspase-8 or procaspase-10 to the complex, where the caspases are activated [160]. BCL-2 homology proteins, with the notable exception of BID [198,199], play a minimal role in regulating death receptor-mediated PCD [160]. Instead, FLICE inhibitory proteins (FLIPS), which mimic procaspase-8 structurally but have no active site for death effector domain-induced activation, are important regulators of extrinsic PCD [200]. FLIPS compete with death receptor signal transduction at the adaptor protein level by binding to the activating regions in the cytoplasmic death domain sites of FADD/TRADD and competing with signal transduction for activation of caspase-8 [160]. Additional regulators of extrinsic PCD include components that exert effects on the signaling complex, such as receptor-interacting protein (RIP) and TNF receptor-associated factor-2 (TRAF2); RIP and TRAF2 interact with the ubiquitous cellular inhibitor of apoptosis proteins (IAPs) [34,201]. IAPs can also prevent PCD induced by external stimuli by directly binding to and inactivating caspases [177]. Decoy receptors, which compete with death receptors for binding of apoptotic ligands such as FASL and TNF-related apoptosis-inducing ligand (TRAIL), also regulate extrinsic PCD [34]. As with intrinsic PCD, various pathogenic bacteria and their products manipulate extrinsic PCD pathways in eukaryotic cells.

APOPTOSIS-REGULATORY MOLECULES OF GRAM-POSITIVE BACTERIA

Pathogenic bacteria use several strategies to circumvent normal immune responses, including seizing control of apoptosis machinery [202]. Indeed, the number of bacterial pathogens shown to exploit PCD pathways in human cells for their own advantage is growing at a rapid pace [203]. Gram-positive pathogens included in this review include species of *Staphylococcus, Streptococcus, Bacillus, Listeria*, and *Clostridium* (Table **1**). These organisms cause serious infections in humans, and all produce toxins or other structural moieties that regulate PCD pathways in eukaryotic cells. The PCD regulatory activities of selected gram-negative bacteria and protozoa have recently been reviewed elsewhere [163,204-206].

An initial comparison of the PCD regulatory activities of gram-positive bacteria with *Shigella flexneri* [2] noted that these organisms induce different forms of PCD in different host cell types. In a similar comparison, intracellular and extracellular pathogens trigger PCD *via* distinct mechanisms [163]. Since these reports, our understanding of the complex interactions between human cells and gram-positive pathogens and their toxins that culminate in or prevent PCD has advanced. It is now clear that different pathways of PCD can be activated by different bacterial species and strains [204]. Thus, PCD is an unexpectedly complex process. Indeed, some forms of cell death that resemble necrosis may involve enzymatic cascades more typical of classical apoptotic cell death [206b]. Here, we discuss microbial triggers of cell death involving hallmarks of apoptotic PCD; activation of initiator and effector caspase cascades,

DNA fragmentation, nuclear/cytoplasmic condensation, disassembly to apoptotic bodies, and non-inflammatory outcomes. It should be noted that many of the models of infection discussed here have not described all of these features of PCD.

Gram-positive bacteria are equipped with an array of virulence determinants (Table **1**). In many of the diseases caused by these organisms, the bacterial toxins produced during infection are largely or wholly responsible for the clinical presentations [207]. Most bacterial toxins act within the cytoplasm of eukaryotic cells, causing host cell dysfunction by affecting ion channels, forming pores in host cell membranes, through protease or nuclease activity, and altering intracellular signal transduction pathways [208]. Other bacterial toxins act at the host cell surface [209]. Bacterial toxins that directly affect PCD [13] can be broadly differentiated into four groups: (1) superantigens (e.g., *S. aureus* enterotoxins, TSST-1 and *S. pyogenes* pyrogenic exotoxins), (2) pore-forming toxins (e.g., *S. aureus* α-toxin, *S. pneumoniae* pneumolysin, *S. pyogenes* streptolysin O, and *L. monocytogenes* listeriolysin O), (3) nonpore-forming toxins with enzymatic activity (e.g., *B. anthracis* lethal toxin and *C. difficile* exotoxins), and (4) non-enzymatic structural components (e.g., lipotechoic acid) [2,23,24, 207].

Staphylococcus aureus

Staphylococcus aureus, a commensal organism that normally colonizes as many as half of healthy adults, is a versatile pathogen that causes community-acquired and nosocomial infections [210]. Exotoxigenic virulence factors produced by *S. aureus* include the pyrogenic superantigen enterotoxins A through E, G, H, and I (not superantigenic), toxic shock syndrome toxin-1, the hemolysins α-toxin, β-toxin, δ -toxin, and γ-hemolysin, the exfoliative toxins epidermolytic toxins A and B, and Panton-Valentine leukocidin [207,210,211]. These exoproducts are robust and stable to chemical inactivation, proteolysis, and heat [211,212]. Other secreted products include protease, lipase, and hyaluronidase. Membrane-spanning endoproducts on the *S. aureus* surface include fibronectin-binding protein, collagen-binding protein, and elastin-binding protein, the B-lymphocyte superantigenic toxin protein A [213], coagulase, and clumping factor. Finally, the peptidoglycan cell wall and capsule of *S. aureus* also contribute to human infection [210].

Whole live *S. aureus* induces PCD in monocytes [214,215], chondrocytes [87], keratinocytes [88,89], endothelial cells [81,216], epithelial cells [217,218], and osteoblasts [90]. The ability of an *S. aureus* exotoxin to promote apoptosis and thereby influence immune responses [219] was first reported in enterotoxin B-stimulated murine T cells [37] and human $CD4^+$ T lymphocytes obtained from asymptomatic HIV-infected individuals [38,39]. Superantigens, unlike conventional antigens, interact directly with MHC class II molecules on accessory cells and with particular variable regions of the T-cell receptor β chain (TCRVβ) without the need for prior processing. Direct cross-linking of the TCRVβ to the MHC class II molecule results in polyclonal T-cell activation [220-229] and may ultimately lead to PCD. In this manner, staphylococcal superantigens like enterotoxin B circumvent the normal rules for antigen processing and presentation and trigger the death of specific human T-cell subsets, potentially interfering with the host's ability to respond to conventional antigens. Enterotoxin B has been used extensively as an model to study aspects of tolerance (i.e., deletion and anergy), clonal expansion, and maintenance of peripheral T-cell pools; much is known about its structure and synthesis [211]. Interestingly, there is significant structural homology between enterotoxin B and toxic shock syndrome toxin-1 [211], but only enterotoxin B induces T-cell apoptosis. Toxic shock syndrome toxin-1, in fact, prevents apoptosis in monocytes [76] but induces dose-dependent PCD in B cells [77]. Neither toxin has direct cytotoxic affects on neutrophils, but inhibition of PCD in neutrophils can be brought about by both indirectly as a result of their pro-T helper 2 cell (Th2) stimulatory activity [230]. Many studies have confirmed that

enterotoxin B causes activation-induced cell death (AICD) of T lymphocytes, a process involving sequential T cell receptor (TCR) binding, activation, clonal expansion, FAS expression, and eventual PCD [46,50-55,68]. AICD, which typically involves extrinsic PCD [163], is now recognized as a key process through which effector $CD8^+$ T cells are removed from the lymphocyte pool after disease resolution [231]. AICD caused by enterotoxin B is restricted to activated [44], post-proliferative T cells [43,67], depends on the number of cell divisions [56], and is prevented by costimulation with CD28 [57]. Processes of T-lymphocyte sensitization to PCD after antigen-triggered proliferation are reviewed elsewhere [231] and may differ from those involved in maintenance of lymphocyte homeostasis after microbial infection [130]. Enterotoxin B-triggered fratricidal PCD (i.e., killing of neighboring cells) has also been demonstrated in T lymphocytes [58,232]. Thus, not only does enterotoxin B induce PCD directly in target T cells in which it engages the TCR, but also it causes bystander PCD in proximal lymphocytes in a paracrine fashion. In monocytes, *S. aureus* similarly stimulates the release of soluble FASL from host cells, thereby inducing PCD in infected and bystander host cells [214,233]. Enterotoxin B also induces PCD in human immunoglobulin (Ig)-secreting B cells, a process that occurs in a $CD4^+$ T-cell-contact-restricted, CD95-dependent manner [47]. $CD4^+$ T-lymphocyte-dependent PCD in endothelial cells is also triggered by enterotoxin B [48,49]. Finally, enterotoxin B inhibits PCD in eosinophils [69] and is cleared rapidly after induction of PCD *in vivo* [234].

At the molecular level, enterotoxin B induces TCRVβ-specific PCD in T cells in a manner that mimics normal host-driven, FAS-mediated PCD for peripheral deletion of antigen-expanded T-cell clones [59,60,162,232]. However, whereas ion channels are involved in SEB-triggered PCD in thymic T cells, this is not characteristic of SEB-driven peripheral deletion [235]. The precise series of intracellular signaling events leading to AICD of enterotoxin B-reactive $V\beta8^+$ T lymphocytes is not yet defined, and it should be noted that the role of FAS signaling is controversial [13]. However, mounting evidence of CD95/FASL dependence [59-64, 70-72,161,162] suggests an essential role for extrinsic PCD. Regulation of staphylococcal enterotoxin-triggered PCD might occur at several levels: autonomous FASL-CD95 engagement involving expansion of T lymphocytes and deletion by clonal suicide [59,60,73], alternative regulation by activated $CD8^+$ T cells [74,75], and expression of FASL on nonlymphoid cells as opposed to signal derivation in the activated T cells themselves [232]. FASL-CD95 engagement also appears essential for enterotoxin B-induced PCD in epithelial cells [48]. Other experiments have yielded conflicting results indicating that FAS/FASL signaling has a minimal role in enterotoxin B-stimulated PCD [65]. However, FAS-deficient mice are more susceptible to SEB-triggered lethal shock [236], suggesting that FAS-mediated extrinsic PCD helps to contain overzealous lymphocyte responses during staphylococcal superantigen exposure [13]. FAS-independent mechanisms might compensate for enterotoxin B-triggered, CD95-initiated PCD in experimental systems, where CD95 signaling has been absolutely removed. In mice, FAS-independent PCD in enterotoxin B-stimulated T cells involves decreased expression of BCL-2 and depends on the presence of BIM; this is consistent with the involvement of intrinsic rather than extrinsic signaling [65]. Nitric oxide (NO) appears to exert regulatory influence on enterotoxin B-mediated PCD on $CD4^+V\beta8^+$ T cells *in vivo* [66]. Structure-function relationship studies, similar to those performed to define the structural basis for superantigenicity [211], will help to identify the structural components necessary for the ARM functions of enterotoxin B and toxic shock syndrome toxin-1. A second enterotoxin of *S. aureus* with PCD regulatory activity is enterotoxin A, which induces apoptosis in $V\beta3^+$ T cells and $V\beta11^+$ T cells (but not in $V\beta6^+$ T cells), akin to enterotoxin B-stimulated PCD in $V\beta8^+$ T cells (and $V\beta3^+$ T cells) [50]. The implications of this T cell-subtype specificity and mechanisms are unknown, but interleukin 2 (IL-2) deprivation and *not* CD95-FASL interactions appears to contribute to enterotoxin A-induced PCD in these cells [84]. Interestingly, enterotoxin A-induced apoptosis of T cells does not appear to be MHC Class II-

restricted, as is AICD triggered by enterotoxin E, despite the fact that these exotoxins share more than 80% homology at the amino acid sequence level [85].

S. aureus hemolysin α-toxin, another ARM [79,211], is secreted in monomeric form, penetrates and integrates into the target cell membrane, and forms cylindrical heptamers [211]. Nanomolar concentrations of α-toxin cause pores to form in the membrane of T lymphocytes, and the ensuing fluxes of monovalent ions such as Na^+ (influx) and K^+ (efflux) appear to trigger PCD [13,78]. Alpha-toxin also causes pore formation and PCD in endothelial cells [81,237] and pore formation in neutrophils [238] and neuroendocrine cells [239]; however, hallmarks of apoptotic PCD such as DNA fragmentation and caspase activation have not been demonstrated in these cells. In monocytes, α-toxin induces caspase-dependent PCD [79] that occurs independently of death receptors and involves mitochondrial dysfunction [80]. In keratinocytes, however, α-toxin selectively induces necrosis rather than PCD [240]. This dichotomy reflects the pore size produced by oligomerization of α-toxin monomers, which is concentration-dependent. Smaller pores restrict the passage of larger molecules (e.g., ATP) and divalent ions (e.g., Ca^{2+}) [78], favoring PCD. Toxin concentration dependency has also been reported in other cell types [241]. In contrast to enterotoxin B-induced PCD (i.e., TCR engagement and FASL-CD95 signal transduction), the PCD cascade induced by α-toxin involves caspase activation *via* the intrinsic pathway with mitochondrial cytochrome *c* release and BCL-2 regulation [49,80]. This presents an interesting scenario where two distinct ARMs, namely enterotoxin and hemolysin, from a single organism exert different effects on host cells and trigger distinct forms of PCD. A more recent study suggested that α-toxin can also induce an unusual form of necrosis in T cells that involves coincidental caspase activation [242].

Finally, the B-cell superantigenic protein A of *S. aureus* induces dose-dependent PCD in murine lymphocytes by altering the concentration of BAX [82]. Ligation of the antigen receptor in B cells induces dissipation of mitochondrial membrane potential and activation of caspases, a process regulated by BCL-2, but independent of CD95/TNF receptor [83]. Thus, similar to PCD induced by α-toxin, that induced by protein A appears to trigger intrinsic PCD. Earlier studies in murine cells demonstrated that protein A-induced B-cell proliferation involves the overexpression of BCL-2 [243], a process associated with increased cell survival in some experimental systems [244] and concomitant Th1 stimulatory activity [245]. It is not known if the effects of protein A on human cells are similar to those observed on murine cells.

Streptococcus spp

Streptococcus agalactiae—*Streptococcus agalactiae*, or Group B Streptococcus (GBS), is a common cause of pneumonia, sepsis, and meningitis in newborns, pregnant women, and adults with underlying medical conditions [246]. Neonates acquire GBS at birth from their asymptomatically colonized mothers (35%-40% of pregnant women have lower gastrointestinal or genitourinary GBS colonization) [247,248]. The neonatal lung may be infected with a substantial inoculum of GBS at birth, because the bacteria can grow to high numbers in human amniotic fluid [246]. Therefore, the proficiency of the antibacterial function of newborns' alveolar macrophages [249-251] is considered a crucial component of early immune responses against GBS [252]. Pulmonary epithelial and endothelial cells, and neutrophils also respond to GBS [246,253]. GBS persist inside macrophages for an extended period of time after nonopsonic phagocytosis and eventually trigger PCD in macrophages and monocytes [91,92,254,255]. Intracellular persistence and manipulation of PCD pathways and antibacterial activity in macrophages may represent an important virulence mechanism whereby GBS contributes to the characteristically poor inflammatory response in the neonatal lung. GBS-induced PCD is also a prominent feature of hepatocytes in a rabbit model of GBS sepsis [256] and in neurons of the dentate gyrus in GBS meningitis [93]. PCD in the latter is mediated, in part, by reactive oxygen intermediates [95].

Exotoxigenic virulence factors produced by GBS include hyaluronidase (hyaluronate lyase), CAMP factor, superoxide dismutase, protease, nuclease, platelet-activating factor, and collagenase/oligopeptidase, protein c, RIB, R protein, and C5a peptidase [246,257,258]. The structure of the antiphagocytic capsule also influences pathogenicity (reviewed in [259]). GBS lipotechoic acid is cytotoxic to a variety of human monocytic cell lineages, but the precise form of cell death has not yet been defined. This cytotoxicity may contribute to disease by promoting adhesion and invasion [246]. Among the several toxins produced by GBS, investigations of PCD have focused on β-hemolysin [246,258].

GBS β-hemolysin is bound to the bacterial cell wall and is responsible for the β-hemolysis characteristic of most clinical GBS isolates [246,260]. Under experimental conditions, the β-hemolysin is relatively unstable and difficult to purify, which has hindered its study. PCD induced by GBS infection of macrophages was initially attributed to β-hemolysin, because GBS grown in conditions minimizing β-hemolysin expression, namely high glucose concentrations, were not able to induce PCD in macrophages [91]. The ability of acellular β-hemolysin preparations to induce PCD in macrophages has also been demonstrated [29]. In another study, however, the ability of a β-hemolysin-deficient GBS mutant to induce PCD in macrophages did not differ from that of its hemolytic parental strain, suggesting that PCD can be triggered by additional GBS factors [92].

GBS-induced PCD in macrophages is inhibited by the caspase-3 inhibitor DEVD-CHO [91, 261], and is associated with alterations in BAD, BAX, 14-3-3, and OmiHtr/A2 expression. Alterations in mitochondrial transmembrane potential leading to loss of mitochondrial membrane integrity further indicate involvement of the intrinsic pathway of PCD [261]. Similarly, death of GBS-infected human monocytes is caspase-dependent [94]. NO secretion triggered by GBS infection contributes to PCD in murine macrophages, but similar findings have not been shown in human macrophages, suggesting an alternative NO-independent mechanism in human cells [262]. Interestingly, TNF-α is required for GBS-induced PCD in neurons [93], but appears dispensable for GBS-induced PCD in macrophages [262]. Oxygen intermediates also contribute to GBS-induced PCD in neurons *in vivo* [263]. GBS triggers increases in intracellular Ca^{2+}, and activates selected mitogen-activated protein kinases (MAPKs) including c-Jun NH_2-terminal kinase and p38 [264]. In monocytes, GBS causes up-regulation and secretion of TRAIL, which is associated with PCD [94], although functional studies using TRAIL depletion or deletion have not been reported. GBS also induces phosphatidylserine exposure, which probably contributes to the clearance of the dying apoptotic cells [265]. Investigation of GBS ARMs is in its infancy and further study of the effects of GBS products on eukaryotic cells will be important to define how GBS-triggered PCD influences disease pathogenesis and immune responses.

Streptococcus pneumoniae—*Streptococcus pneumoniae* is part of the normal microflora of the human respiratory tract and a common cause of respiratory infections and meningitis [101,266]. Pneumococcal virulence, like that of GBS, is in large part determined by the chemical composition and synthesis of its capsular polysaccharide [101]. *S. pneumoniae* expresses several additional virulence factors, as reviewed elsewhere [267], that may initiate PCD in phagocytes after microbial uptake. Pneumolysin [268,269] has direct proapoptotic activity on several cell types including microglia, neurons [97,99], and macrophages [98] but not on epithelial cells [105]. Other factors produced by *S. pneumoniae*, i.e., super oxide dismutase, NADH oxidase, zinc metalloproteinases, and H_2O_2, also contribute to virulence [101], but among these, only H_2O_2 has proapoptotic activity toward human cells [97]. Surface endoproducts of *S. pneumoniae* include the capsule, hyaluronate lysase, pneumolysin (classified here as a surface molecule that is also secreted), neuraminidases, autolysin, choline-binding protein A, pneumococcal surface antigen A, lipotechoic acid, LPXTG-anchored proteins (e.g., surface protein A and surface protein C), and other lipoproteins [270]. In one

study, pneumococcal cell wall extracts induced PCD in monocytes [100]; however, with the exception of pneumolysin, there are no reports linking apoptosis to individual highly purified *S. pneumoniae* endoproducts.

S. pneumoniae stimulates PCD in a variety of human cell types. Whole live *S. pneumoniae* initiates PCD in dendritic cells by two distinct mechanisms [101] and NO-dependent PCD in monocytes, macrophages [98] and neutrophils [102-104]. Pneumococcus also induces PCD in human alveolar and bronchial epithelial cells [105,107] and in neurons [97]. Increased numbers of apoptotic T cells have been reported in patients infected with *S. pneumoniae.* [106]. In an animal model of *S. pneumoniae* infection, inhibition of PCD in lymphocytes in pneumococcal septicemia improved host survival, a finding that suggests that T-cell PCD triggered by the bacterium is detrimental to effective immunity [108,109]. The majority of studies on pneumococcus-induced PCD, however, have focused on macrophages because of their crucial role in early immune responses and ability to bridge innate and adaptive immunity through pathogen-associated molecular pattern recognition [271,272].

PCD in *S. pneumoniae*-infected macrophages requires internalization of bacteria and continued macrophage protein synthesis [9]. This differs from GBS-induced PCD of macrophages, where external signal transduction resulting from bacterial attachment to the macrophage surface can result in PCD without bacterial internalization [91]. The concept that a particular ARM of *S. pneumoniae* instigates PCD in macrophages to facilitate immune escape is not entirely supported by the ultimate intracellular fate of the bacterium, i.e., death, as discussed elsewhere [9]. Indeed, inhibition of pneumococcus-induced PCD *in vivo* promotes bacterial dissemination and implicates PCD as a crucial component of an immune response that helps to contain and eliminate *S. pneumoniae* [273]. Indeed, opsonization enhances pneumococcus-induced PCD in macrophages by increasing the number of intracellular bacteria [100]. In contrast, recent data on GBS have indicated that, unlike *S. pneumoniae*, a proportion of intracellular GBS can avoid intracellular killing and persist inside apoptotic macrophages, which may facilitate bacterial escape from infected cells [92,262]. Therefore, host-driven PCD in macrophages appears to contribute to immune control of pneumococcus, but PCD may play a distinct role in infections caused by GBS.

Pneumolysin, like β-hemolysin of GBS, is a pore-forming exotoxin [268,269]. Similar to *S. aureus* α-toxin, pneumolysin inserts into the host cell membrane, where it forms pores [274]. The role of pneumolysin in PCD was initially unclear, because early observations indicated it inhibited bacterial phagocytosis and prevented PCD in macrophages [9]. More recent genetic studies have confirmed that pneumolysin triggers PCD in macrophages [98] and other cell types [97]. *S. pneumoniae*-induced PCD occurs by FAS-independent mechanisms in infected monocytes and by FAS-dependent mechanisms in noninfected bystander monocytes [9]. In macrophages, the PCD response is associated with mitochondrial membrane permeabilization, translocation of cytochrome *c* to the cytoplasm, and downregulation of the antiapoptotic BCL-2 family membrane protein myeloid cell leukemia sequence (MCL-1) [98]. BAX and BID may also contribute to *S. pneumoniae*-induced PCD in human macrophages [98]. Interestingly, encapsulated *S. pneumoniae* does not induce PCD in macrophages as well as nonencapsulated bacteria, suggesting that the capsule may cloak pneumococcal ARMs and reduce their effect. Alternatively, reduced PCD in response to encapsulated pneumococcus may reflect impaired bacterial uptake, since internalization is required for *S. pneumoniae*-induced PCD [9,100]. Caspase-3 is required for *S. pneumoniae*-induced PCD in macrophages, similar to PCD triggered by GBS [9].

Pneumococcus-induced PCD in neurons involves different mechanisms than those responsible for PCD of macrophages or monocytes [204]. Neuronal PCD results from rapid H_2O_2-mediated mitochondrial damage, release of apoptosis-inducing factor (AIF) [275], and intracellular

Ca^{2+} fluxes with [276] or without caspase activation [97,277,278]. Pneumolysin contributes directly to these processes [97], whereas the capsule does not [97,275]. *S. pneumoniae*-infected pulmonary epithelial cells undergo PCD through mechanisms that depend on caspase-6, -8, and -9 but not caspase-3, and result in cleavage of BID and BAX and downregulation of BCL-2 [105]. As with neurons, PCD in parenchymal cells of the lung is independent of the pneumococcal capsule [105].

Streptococcus pyogenes—*Streptococcus pyogenes*, or Group A Streptococcus, is the etiologic agent of scarlet fever, necrotizing fasciitis, impetigo, and various postinfectious autoimmune sequelae [279-281]. *S. pyogenes* colonization of the skin or nasopharynx precedes local and invasive infection, and the site of infection is related to the expression of specific *S. pyogenes* virulence factors [281]. Secreted exotoxigenic virulence factors produced by *S. pyogenes* include the pore-forming β-hemolysin toxins streptolysins O and S, DNases A through D, hyaluronidase, NADase, streptococcal inhibitor of complement, various streptococcal superantigenic pyrogenic exotoxins (A-C, F-H, J-L, SmeZ, SmeZ-2), streptococcal pyrogenic exotoxins I and M, exotoxin F (mitogenic factor), the cAMP cohemolysin, and streptokinase [281-283]. Streptococcal pyrogenic exotoxin A shares homology with *S. aureus* toxic shock syndrome toxin-1 and a similar mechanism of T-cell activation [284]. Streptococcal pyrogenic exotoxin B, on the other hand, shares significant homology with *S. aureus* enterotoxins A and C and has cysteine protease activity that cleaves immunoglobulins, fibronectin, vitronectin, extracellular matrix proteins, histamine, and IL-1β [282,283]. Surface moieties and endoproducts of *S. pyogenes* include lipotechoic acid, the outer hyaluronic acid capsule, M protein, protein F, multiple fibronectin-binding proteins, serum opacity factor, C5a peptidase, GAPDH, and vitronectin-binding, galactose-binding and collagen-binding proteins [281-283]. Several *S. pyogenes* exotoxins have been characterized in detail; however, the knowledge on PCD induced by *S. pyogenes* is limited.

In contrast to other pathogenic *Streptococcus* spp., *S. pyogenes* induces large-scale necrosis of a range of host cells. Widespread necrosis, inflammation, and vascular permeability is characteristic of severe *S. pyogenes* infections [112,285] and is linked to the exotoxins and endoproducts expressed by the organism. Several studies have suggested a role for *S. pyogenes*-induced PCD in the infectious process. In experimental models of *S. pyogenes* infection, whole live *S. pyogenes* induces PCD in human neutrophils [115], keratinocytes [110], and epithelial cells [22,111,286,287]. With the exception of one study in patients with severe *S. pyogenes* infection in which *S. pyogenes*-induced PCD was associated with depletion of specific T-lymphocyte subsets expressing particular Vβ regions [288], the occurrence of PCD in patients infected with *S. pyogenes* has not been reported. In keratinocytes, *S. pyogenes*-induced PCD requires delivery of secreted bacterial NADase into the host cell cytoplasm, where it acts as an intracellular cytotoxin. This process appears to depend on prior streptolysin O-mediated pore formation in the host cell membrane, which facilitates NADase transfer [110]. Thus, it appears that NADase and streptolysin O act as cooperative ARMs of *S. pyogenes* for PCD in some cells.

Streptococcal pyrogenic exotoxin B enhances PCD in epithelial cells [111] and macrophages [113] by a mechanism that may involve cleavage and activation of an IL-1β precursor [289]. Recently, Tamura *et al*. (2004) demonstrated that the proapoptotic activity of streptococcal pyrogenic exotoxin B is associated with the ability of the toxin to activate eukaryotic matrix metalloproteinases that cause the release of TNF-α and FASL and activate PCD [112]. Nakagawa *et al*. (2004) demonstrated that *S. pyogenes* triggers caspase-9 activation in epithelial cells resulting in dysfunctional permeability transition pores and mitochondrial cytochrome *c* release [286]. Finally, BAX translocates to the mitochondria, and the prosurvival proteins BCL-2 and BCL-X_L are depleted [22]. These observations implicate intrinsic PCD in *S. pyogenes*-infected epithelial cells. The fibronectin-binding F1 surface protein of *S. pyogenes*

appears to be essential for the organism to invade and induce PCD in epithelial cells [22]. One study investigating the contribution of M protein to host cell PCD responses indicated that PCD after *S. pyogenes* infection might be influenced by the specific M protein serotype [22]. Finally, purified glycoprotein of *S. pyogenes* induces PCD in fibrosarcoma cells *via* mechanisms that appear to involve inhibition of tyrosine kinases and dephosphorylation of host proteins [114].

The marked necrosis and severe inflammation characteristic of *S. pyogenes* infections suggest that PCD may play a minimal role in disease pathogenesis. These characteristics of infection, however, do not preclude involvement of PCD in the host cell response to *S. pyogenes*. It appears plausible, for example, that selected pyrogenic exotoxins of *S. pyogenes* might expand particular T-cell subsets and cause AICD in a manner that parallels lymphocyte-pathogen interactions described for *S. aureus* exotoxins. Current studies demonstrate that *S. pyogenes* can directly manipulate particular apoptotic programs in several human cell types, and future studies will elucidate what role these interactions play in the infectious process.

Bacillus anthracis—The host responses involved in *Bacillus anthracis* infection are reviewed elsewhere [290,291]. In inhalational anthrax, alveolar macrophages phagocytose inhaled bacterial spores but are incapable of efficiently killing the organism. Indeed, macrophages may facilitate spread of the bacteria by carrying it to regional lymph nodes [291,292]. Spores germinate in regional lymph nodes, producing vegetative bacteria that cross endothelial cells to the bloodstream [293,294]. *B. anthracis* produces a number of exotoxins, collectively known as the anthrax “toxin complex” [293]. The complex is comprised of three polypeptide subunits; lethal factor, edema factor, and protective antigen [291,295-297]. Separately, none of these protein subunits are toxic [293]. Instead, the lethal factor and edema factor form A-B binary units, and when paired with the nontoxic protective antigen, they produce lethal toxin and edema toxin, respectively [290,291,293]. Protective antigen mediates entry of the dimeric toxin into the host cell cytosol [290] by forming protective antigen self-heptamers at the host cell membrane; these self-heptamers bind specifically to the anthrax toxin receptor, which is ubiquitous on host cell surfaces [298]. This receptor-heptamer complex permits the subsequent docking of lethal factor or edema factor and the transfer of the cytotoxic components into the cytosol *via* receptor-mediated endocytosis [291,293,296]. Preassembled toxin complexes may also bind directly to the receptor [295]. A second receptor for anthrax toxin was recently identified [299]. Additional *B. anthracis* exoproducts and endoproducts include the surface-layer protein SAP (secreted and cell-bound) [291], the antiphagocytic poly-γ-D-glutamic acid capsule, the surface-layer protein EA1, and cell wall structural polysaccharides and peptidoglycan [291,300,301]. Lipotechoic acid and membrane lipoproteins of *B. anthracis* have not yet been described, although these products are present in other *Bacillus* spp. [301].

Necropsy of anthrax victims demonstrates apoptotic cells in tissues of the lower respiratory tract [302]. Virtually all types of eukaryotic cells studied to date are sensitive to edema toxin, which is an adenylate cyclase [290,291,295]. Edema toxin causes cytokine deregulation and impaired phagocytic cell function by increasing intracellular cAMP [290,293]. Lethal toxin, on the other hand, is a metalloproteinase that can replicate the hypotension, shock, and death caused by systemic infection and appears selectively toxic to macrophages [2,26,290,291, 294]. Macrophage specificity is thought to reflect the presence of a unique intracellular target within these cells or a unique form of toxin processing that activates the lethal toxin after internalization [293]. Compared with rodent macrophages, human macrophages are relatively resistant to the actions of lethal toxin; therefore, PCD in these cells probably has a more limited role in the pathology of infection in humans [303]. However, under certain conditions, human macrophages do undergo lethal toxin-induced PCD [25,116,117,304,305], which may contribute to escape and dissemination of the organism from these cells [303].

Reports about the effect of interferon-γ (IFN-γ) on lethal toxin-induced PCD in human macrophages have been conflicting. Popov *et al*. (2002) reported a sensitizing effect leading to increased cell death [116]; however, Gold *et al*. (2004) described a prosurvival effect [304]. TNF-α may also sensitize macrophages to the cytotoxic effects of lethal toxin [117]. Lethal toxin also induces PCD in human neutrophils [116] and immunologically incapacitates, rather than kills, dendritic cells, thereby rendering them inefficient as antigen presenters and T-cell activators [306]. Two recent studies have demonstrated that the proapoptotic activity of lethal toxin toward human endothelial cells involves decreased BCL-2 [118] and caspase activation [119]. Although generally reported as nontoxic, protective antigen can induce PCD in human monocytes under some conditions [116].

The mechanisms responsible for the proapoptotic activity of lethal toxin have been studied in murine macrophages and *in vivo* models of inhalation anthrax. Lethal toxin-induced PCD is associated with FASL binding and activation of caspases-1, -3, -4, and -8 [26,116]. In mice challenged with *B. anthracis*, administration of a pan-caspase inhibitor improved survival; thus, PCD appears to contribute to severe anthrax [307]. At high concentrations, lethal toxin triggers substantial production of reactive oxidants, causing macrophage necrosis [293]. Necrosis and PCD can also occur simultaneously in cells treated with low doses of lethal toxin [120,121,308]. Macrophage PCD induced by low concentrations of lethal toxin dampens local immune responses and impairs innate immunity [116]. Lethal toxin inhibits MAPK kinases (MKKs) and inactivates MAPKs [25,290,309-312]. The later is essential to PCD triggered by lethal toxin [25,309,313], as these signaling molecules promote survival by inducing NFκB and the transcription of related genes, such as those encoding IAPs [314,315]. Signal transduction through Toll-like receptor 4 and repression of antiapoptotic p38 MAPK, in a process that requires the protein kinase PKR, are particularly important to the ability of lethal toxin to trigger PCD in macrophages [122]. The rapidly expanding body of knowledge on anthrax and the mechanisms of *B. anthracis* toxicity will further define how these molecules direct human cells through death pathways. It will be particularly important to define the PCD responses of host cells to *B. anthracis* in the context of combined treatment with lethal toxin, edema toxin, and other toxins, because these combinations are likely to occur in systemic anthrax infection.

Listeria monocytogenes—The incidence of human listeriosis is relatively low compared to the large number of patients afflicted each year with staphylococcal or streptococcal infections. The overall mortality rate, however, is much higher for listeriosis [316-318]. Listeriosis is a foodborne illness that is most often characterized by gastroenteritis, meningitis, encephalitis, or sepsis [317,318]. *L. monocytogenes* is capable of persistent intracellular survival and replication in epithelial cells, endothelial cells, dendritic cells, fibroblasts, and macrophages [317]. Hepatocytes represent the principle site for intracellular replication [319], although macrophages may also be an important reservoir [320]. In fact, *L. monocytogenes* can completely avoid the extracellular environment within the host by moving from cell to cell *via* polarized actin polymerization-dependent mechanisms [316,317,321]. Thus, *L. monocytogenes* is often regarded as the prototypical organism for study of intracellular pathogenesis [316,321]. A detailed picture of the molecular events involved in the organism's intracellular lifestyle has been compiled and has provided insight into how *L. monocytogenes* modulates eukaryotic host cell function. The PCD regulatory activities of *L. monocytogenes* are only now beginning to be elucidated.

L. monocytogenes attachment, phagocytosis/invasion, intracellular survival, replication, and intercellular trafficking are mediated by distinct virulence factors. Virulence determinants [316,322] and their role in immunity [323,324] are reviewed elsewhere. Despite intracellular replication of the bacteria, most types of eukaryotic cells are not rapidly killed by *L. monocytogenes* [325]. This suggests that PCD has a role in immunity. Indeed, the organism

itself uses multiple mechanisms to *avoid* host cell cytotoxicity [325], perhaps as a means of sustaining its intracellular habitat. Genetically engineered *L. monocytogenes* that kill host cells prematurely, for example, are avirulent compared to their wild-type parent strains [325]. The *Listeriae*, in effect, are shielded from extracellular immune factors whilst they remain intracellular [325]. Bacilli released from dead host cells into the extracellular environment may be targeted for destruction by neutrophils and humoral components [326]. Host-driven PCD, therefore, might benefit immunity [327]. This premise, however, is based on the assumption that all host cell types play similar roles in immunity, which is not the case. Antigen-presenting dendritic cells, for example, are probably not a significant reservoir for *L. monocytogenes* [320] but are critical for induction of T-cell responses. Moreover, *Listeriae* may preferentially take advantage of selected cell types such as hepatocytes for survival and intracellular growth. These facets lend support to the theory that the requirements for successful immunity against a pathogen residing preferentially in macrophages are likely to differ from those immune-effector mechanisms required to combat a pathogen residing in parenchymal cells [326]. Thus, it is not surprising that *L. monocytogenes* infection is associated with PCD in several types of host cells, depending on the stage of infection.

Apoptosis in response to infection with whole, viable *L. monocytogenes* has been described in hepatocytes [328-330], epithelial cells [128], $CD4^+$ T cells, $CD8^+$ T cells, and B lymphocytes [125,130,134], neurons, Purkinje cells [127], dendritic cells [124,331], and natural killer (NK) cells [129] but not in macrophages [8,124,332]. Whether intracellular *Listeriae* actively impede PCD in macrophages to preserve their intracellular niche is an interesting and unresolved question [333]. Macrophages die by delayed necrosis after extensive intracellular bacterial replication [334].

Activation of PCD in infected hepatocytes could contribute to resolution of disease in two ways: First, this eliminates infected cells and reduces the available pool of target host cells for intercellular replication and spread of infection [316,334]. Second, PCD would expose the bacteria to the extracellular milieu, where they are susceptible to extracellular effectors of immunity [319,335]. Neutrophils may mediate hepatocyte death and abort the cycle of cell-to-cell spread [13,326,336], though PCD may also occur in hepatocytes in the absence of neutrophils [328,337]. Caspase-3 appears to contribute to PCD in hepatocytes, but without cleavage of the typical caspase-3 substrate PARP [319]. Selective host-mediated depletion of activated non-pathogen-specific T lymphocytes during the early phase of listerial infection [338] might allow for maximal expansion of the antigen-specific T-cell compartment [318], which could bolster the rapidity and intensity of the adaptive response. PCD in post-proliferative T lymphocytes, after resolution of active listeriosis, may maintain only enough memory T cells required for an efficient response to reinfection, thereby contributing to the regulation of homeostatic lymphocyte pools [130].

In contrast to these potentially immune-beneficial scenarios, *Listeriae*-induced PCD in antigen-presenting cells such as dendritic cells may reduce numbers of professional phagocytes, diminishing antigen presentation and hindering efficient adaptive immune responses [124, 316,334]. *Listeriae*-induced PCD in dendritic cells can occur independently of bacterial internalization [13,124]. Similarly, *Listeriae*-driven PCD in specific subsets of lymphocytes, such as $CD8^+$ T cells, would eliminate important adaptive mediators before the resolution of infection [27,123]. Direct evidence of a detrimental role of PCD in immunity to listeriosis was provided by Kagi *et al*. (1994) who demonstrated decreased innate and $CD8^+$ T lymphocyte-driven cell-mediated immune responses to *L. monocytogenes* in perforin knock-out mice [339] (perforin is integral to host cell-driven PCD pathways in listeriosis). Merrick *et al*. (1997) demonstrated that more severe infection triggers PCD in a nonspecific manner in T and B lymphocytes; this nonspecific response could also hinder adaptive responses [134]. These observations of PCD in B cells are important in light of new data [340,341] that challenge the

long-standing belief that B cells do not contribute to immunity against listeriosis [316,325, 342].

Caspases-1 and -11 do not appear to contribute to *Listeria*-induced PCD in the cell types studied to date [319,343]. In contrast, perforin and IFN-γ have been implicated [344]. Several studies have demonstrated that cytotoxic $CD8^+$ T lymphocytes kill *Listeria*-infected hepatocytes by perforin-granzyme-mediated PCD [132,319,339,345] (early), FAS-FASL-mediated PCD [346-348] (later), or both. This is believed to be an important acquired immunologic effector mechanism [349]. Cytotoxic NK cells might also kill infected hepatocytes by similar mechanisms [319]. TNF-α appears to be crucial in directing a separate, $CD8^+$ T-lymphocyte-mediated PCD pathway in hepatocytes in the absence of perforin and FAS [350]. Miura *et al*. (2000), however, reported that TNF-α and IL-6 play a protective role in mediating survival of hepatocytes during listeriosis [329]. In support of this finding, pancreatic cells in mice lacking TRp55, a key receptor for TNF-α, are prone to PCD triggered by *L. monocytogenes* [351].

The most widely studied exotoxigenic virulence factor of *L. monocytogenes* is the pore-forming hemolysin listeriolysin O (Hly in earlier studies) [316]. Other secreted virulence factors include the phospholipases A and B, which are involved in escape from the phagosome [316]. There are approximately 140 surface proteins of *L. monocytogenes* [316,352]. Only listeriolysin O, which is similar in structure and function to Group A streptococcal streptolysin O [316], and possibly phospholipase C [353,354], modulate PCD in host cells. Listeriolysin O promotes PCD in T cells [27,123] and dendritic cells [124], thereby negating the need for bacterial internalization for PCD of the host cell. A recent study by Menon *et al*. (2003) implicated listeriolysin O as the major virulence factor directing PCD in B cells [125]. The PCD activity of listeriolysin O, like that of *S. aureus* α-toxin [78] and *S. pyogenes* streptolysin O, is dose dependent. This characteristic may affect the disease process in distinct ways. For example, severe disease associated with higher bacterial burdens (more exotoxin) might cause inflammatory necrotic pathology, whereas lower bacterial burdens (less toxin) cause PCD [124].

Functional aspects of listeriolysin O activity are discussed elsewhere [316]. Unlike other bacterial poreforming toxins, listeriolysin O is not cytotoxic under normal conditions. This is believed to be related to the limited pH range in which the toxin is active (pH 4.5-6.5); thus, listeriolysin O activity is compartmentalized to acidified vacuoles [316,325]. Listeriolysin O-induced PCD in T lymphocytes is associated with the activation of caspases-9 and -3 and loss of mitochondrial membrane potential [27], pointing to intrinsic PCD. In addition, listeriolysin O stimulates caspase-independent PCD mechanisms [27], which have not yet been identified. Most T lymphocytes undergoing apoptosis in response to *L. monocytogenes* and listeriolysin O are activated/postproliferative [27,56,67,130]. Removal of γδ T cells, which link innate and acquired effector mechanisms [355,356], early during low-grade infection has been associated with FAS-FASL death receptor signaling [131]. However, FAS-dependent PCD appears to play a more prominent role in the elimination of T cells after resolution of infection [132, 133,206]. Ironically, γδ T cells are also thought to be important in the induction of PCD in activated macrophages to aid in resolution of inflammation [357]. In murine listeriosis, TRAIL contributes to PCD in lymphocytes and myeloid cells during the acute phase of infection, and TRAIL-deficient mice are more resistant to infection than their wild-type counterparts [358]. These observations suggest that TRAIL-mediated PCD represents an important *Listeriae*-driven mechanism for depletion of cells that contribute to immune responses. Type I IFNs contribute to PCD in splenocytes [126], macrophages [359], and T cells [123] in response to listeriolysin O demonstrating the importance of cytokines induced by the organism [360-362]. Finally, high levels of expression of CD94 limit PCD in $CD8^+$ T lymphocytes, which may maintain T-cell pools during infection [129]. Whether these described apoptotic

processes contribute to disease pathogenesis or host defense remains unknown [333]. Clearly, PCD in listeriosis is emerging as a complex, multifaceted process that will require careful investigation.

Clostridium spp

The genus *Clostridium* comprises approximately 20 species known to be pathogenic to humans including, most notably, *C. difficile, C. perfringens, C. botulinum*, and *C. tetani*. As a group, the *Clostridia* produce a vast number of toxigenic products ranging from mild cytolysins to the most toxic biological substance known, the botulinum paralytic neurotoxin [297,363]. PCD regulatory activities and ARMs have been studied in detail in *C. difficile*, the most important cause of nosocomial diarrhea in adults [364-368], and *C. perfringens*, a common agent of gas gangrene and food poisoning [363].

C. difficile produces several secreted and surface-bound virulence factors including flagellae, adhesins, hydrolytic enzymes, an ADP-ribosylating toxin [369], and an anti-phagocytic polysaccharide capsule. After ingestion, acid-resistant spores facilitate survival and allow the organism to colonize the lower intestinal tract [364,367]. The best-characterized virulence determinants of *C. difficile* are the proteinaceous exotoxins A and B [366]. These toxins, which are similar in function and mechanism of action, are largely responsible for the clinical syndromes of diarrhea and colitis [367], and both exert PCD regulatory activity. Immunity to *C. difficile* infection is conferred by specific anti-exotoxin immunoglobulin [364,367]. Colonic epithelium appears to be the main target of exotoxins A and B [365].

Typically, *C. difficile* antibiotic-associated diarrhea is characterized by extensive colonic inflammation and widespread necrosis [364,365,368]. Exotoxins A and B stimulate necrosis in certain cell types (e.g., enterocytes and monocytes) [365,370-373] and are thought to contribute to this pathology. The toxins also cause degranulation in mast cells and induce reactive oxygen metabolites [374-377]. Initial studies on exotoxin A by several groups [378-382] and on toxin B by two groups [383,384] described toxin-induced cytopathology in various cell types; however, those actions have since been studied mostly in enterocytes. After binding to receptors on enterocytes and subsequent endocytosis, the toxins cause disaggregation of actin in colonocytes, and glucosylate or inactivate Rho family signaling proteins. These GTPases, which regulate the formation of actin cytoskeleton stress fibers and act as molecular switches, control various signal transduction pathways, including NF-κB activation and the expression of apoptosis regulators such as BCL-2 [209,385,386]. This leads to epithelial cell rounding and opening of epithelial cell tight junctions. Second, the toxins induce the release of cytokines by activating MAPK [365-367,374]. Loss of tight junction integrity further leads to detachment and rounding of host cells, a known stimulus of PCD [365,367,374,387,388].

Exotoxins A and B trigger PCD in several epithelial cell types. Mahida *et al.* (1996) provided the first direct evidence of toxin A-triggered PCD in intestinal enterocytes [135]. Other reports of PCD-stimulating activity of toxin A towards mast cells, hepatocytes [138], and colonic lamina propria cells (T lymphocytes and eosinophils) [135-137] and endothelial cells [148] followed. Toxin B-induced PCD in intestinal epithelial cells was subsequently demonstrated [144]. *C. difficile* toxin-induced PCD regulatory activities have since been studied by several groups [143,389-391]. Within minutes of endocytosis, the toxins cause a decrease in ATP [139,140], a loss of mitochondrial membrane potential [141], the release of mitochondrial cytochrome *c*, and the generation of toxic reactive radicals [139,142]. Activation of NF-κB is believed to be involved in these responses [374,376]. Mitochondrial dysfunction occurs before a decrease in Rho protein activity [374], which appears necessary for PCD [139]. This is consistent with Rho protein prosurvival signaling in other systems [392-394]. Activation of caspases-1, -3, -6, -8, and -9 [141] indicates extensive procaspase cleavage culminating in

activation of both the intrinsic and extrinsic PCD pathways. In endothelial cells, *C. difficile* toxins activate intrinsic PCD *via* alterations in BCL-2, MCL-1 and BID [148]. Toxin B-induced PCD in epithelial cells is also associated with Rho protein dysfunction and inhibition of cell adhesion due to actin depolymerization [144,366]. Toxin A also activates BID [139], which may link the intrinsic and extrinsic PCD pathways. TNF-α and FASL do not appear to be involved in the early pathway of toxin A-triggered PCD [137]. Toxin B has been used to stimulate apoptosis in various systems [389,391]. Like toxin A, toxin B activates caspase-3, causes PARP cleavage [145], and reduces BCL-2 levels [395], in a pathway that may be p53-dependent [146]. The proapoptotic effects of toxin B can be reversed by host cell MAPK activity, under certain conditions [143,145]. In addition, caspase-independent pathways triggered by toxin B exist, and these also appear to involve inactivation of Rho proteins [147].

C. perfringens, unlike *C. difficile*, produces an array of toxins implicated in eukaryotic cell cytotoxicity including enterotoxin, collagenase, hyaluronidase, sialidase, pore-forming θ-toxin (perfringolysin), α-toxin (phospholipase), β-toxin, ε-toxin, and τ-toxin [208,363,396,397]. Enterotoxin induces PCD by binding to as yet unidentified enterocyte surface receptors and activating caspases-3 and -7, causing mitochondrial cytochrome *c* release, extracellular Ca^{2+} influx [150], and eventual host cell death [149], which is dose-dependent [150,398]. Although β-toxin appears to be an oligomerizing pore-forming toxin that permits movement of cations through susceptible membranes, no studies have addressed its role in PCD [399]. Similarly, although the α-toxin has cytotoxic activity [400], no PCD regulatory activity has been reported.

Collectively, studies on different *Clostridia* spp. suggest that the intrinsic pathway of PCD involving mitochondrial dysfunction and inactivation of Rho protein function is a common mechanism by which distinct exotoxins from these organisms trigger PCD in host cells [209]. Exoenzyme C3, the major exotoxin of *C. botulinum*, also exerts inhibitory activity on Rho GTPases and causes PCD in host cells such as lymphocytes [388,392]. Whether *C. perfringens* enterotoxin targets Rho proteins in the host cell is an important unresolved question. It is also important to note that additional non-Rho protein-related pathways of PCD are stimulated by clostridial toxins, as exemplified by *C. sordellii* lethal toxin [401]. The contribution of actin cytoskeleton disruption brought about by toxin-mediated inactivation of Rho GTPases to these apoptotic processes is at present unclear [209]. What role the PCD plays in immunity to the different infections caused by *Clostridium* spp. remains to be determined; however, several possible influences on immunity can be predicted.

During *C. difficile* infection, intestinal cells undergoing PCD as a result of toxin A exposure could alter the cytokine environment in unique ways. This could sway the balance of helper T cell responses occurring in the lamina propria microenvironment and thereby, affect humoral and cell-mediated immunity. Toxin-induced disruption of colonic epithelium would expose lamina propria T lymphocytes, which activate the suicide response after toxin exposure, and thereby compromise adaptive immunity. Inflammation is a key aspect of the pathologic changes that occur in colitis, and this may be related to toxin dose, i.e., low doses of toxin trigger PCD that may reduce inflammation, and higher doses trigger inflammatory enterocyte death and infiltration. Finally, deletion of mast cells in the lamina propria by toxin-induced PCD could hinder the immune response to infection by affecting the release of histamine, cytokines, and NO [136]. Understanding the role that PCD plays in these infections will be an important step in elucidating the significance of clostridial toxin-induced PCD in human disease. Future use of appropriate animal models will help to address these questions [135, 144].

IMPLICATIONS OF BACTERIA-MODULATED PCD FOR IMMUNITY TO INFECTION

The clinical spectrum of disease caused by the organisms listed in Table **1** is broad; therefore, effective immune responses to these pathogens involve different elements of the immune system. There are a number of crucial aspects of the host-pathogen interaction that are relevant to the process of bacteria-induced PCD and development of immunity. One of these aspects is cellular tropism, or predilection for particular host cell types. Cellular tropism for PCD is demonstrated by a number of the pathogens discussed in this review. For example, the *S. aureus* proapoptotic influence toward lymphocytes and epithelial cells and the prosurvival influence of staphylococcal exotoxins toward eosinophils [69]. Fundamental differences exist in the susceptibility of different cell types to staphylococcal enterotoxin A-triggered PCD [75,211,402]. *Listeria* listeriolysin O induces PCD in T cells and B cells but not in macrophages. Cellular tropism may be influenced by key differences in the ability of the host cell to phagocytose the organism, to produce antimicrobial substances, and to activate second messenger pathways. It is also influenced by the ability of the pathogen to produce bioactive ARMs that can hijack components of the host PCD machinery in a particular cell type. An additional level of complexity in cellular tropism exists when considering that different immune cells exhibit inherent differences in susceptible to apoptosis. For example, NKT cells are relatively resistant to PCD compared to other cell types [403]. Postproliferative T lymphocytes are more susceptible to PCD than are nonantigen-differentiated T cells. Monocytes die by PCD after phagocytosis of various pathogenic bacteria, whereas death of polymorphonuclear leukocytes (PMNLs) is delayed after internalizing the same microbes [404]. These cellular tropisms or “PCD restrictions” may dictate the role of bacteria-induced PCD regulatory activity in the overall immune response during these infections.

The second aspect of the host-pathogen interaction related to PCD activity and immunity is the functional relevance that a particular host cell type has in the overall immune response to the organism. Simply because a pathogen induces (or prevents) PCD under experimental conditions does not mean such an observation reflects a biologically relevant response that occurs during the disease process *in vivo*. It is important to understand the instigator of the PCD deregulation; is it the pathogen or the host that is primarily responsible for triggering the PCD event? In the example of listeriosis, the role of T lymphocytes is to restrict microbial growth by killing bacteria-infected host cells and provide help for antimicrobial sentinels such as macrophages. If PCD in $CD8^+$ T cells during listeriosis is driven proactively by listeriolysin O rather than by the host immune response, our hypothesis would be that PCD hinders adaptive immunity, because $CD8^+$ T cells are key effectors of bacterial clearance. The outcome of these interactions could include deletion of *Listeria*-specific T cells required for immunity and downregulation of the immune system by generation of an immunosuppressive milieu (e.g., less IFN-γ, more TGF-β, and a shift to the Th2 response) [163]. Apoptotic bodies from toxin-killed T lymphocytes could also inhibit the proliferation of antigen-stimulated naïve T cells, which might dampen immune responsiveness [80,405]. Finally, there is the possibility that AICD in T lymphocytes that were triggered by ARMs may result in some surviving T cells with TCRs that become anergic (i.e., lose their capacity to proliferate in response to the antigen *in vitro*), and this could render T-lymphocyte populations suboptimally responsive [51]. T-cell unresponsiveness resulting from interruption of the normal physical interaction between T cells and antigen-presenting cells by apoptotic bodies has also been described; *S. aureus* SEB represents an example of this [406]. On the other hand, T cells would be deleted at the end of active microbial disease, as the host immune-driven homeostatic response returns expanded pools of T-lymphocyte subsets to appropriate levels. Using the example of listeriosis, host immune-driven PCD in infected hepatocytes would have different implications for immunity compared with the affects described for lymphocytes, because these two cell types play very

different roles in the infectious process (i.e., T cells drive immunity and are targeted by the pathogen for elimination, whereas hepatocytes are used as a niche for bacterial survival and are targeted for destruction by the host immune system). Thus, the functional relevance of individual cell types in immunity to a given pathogen bears influence on the potential role of PCD in disease. This must be considered in the context of what comprises a successful immune response to a given organism.

Another potentially important aspect of bacteria-modulated PCD is the immune status of the host and whether different underlying immunologic conditions affect the ability of a pathogen to modulate PCD pathways. To date, few studies have investigated this and little is known about the impact of genetic defects on PCD responses. Studies of immune-deficient animal models, however, have provided some clues. In listeriosis for example, deficient TNF receptor signaling influences the ability of the bacterium to modulate PCD.

From the pathogen's perspective, different bacterial strains within the same species may produce a more or less pronounced PCD event [204]. As a result, our understanding of the significance of PCD regulatory activity of a given species may be incomplete, because some strains trigger (or prevent) the PCD event, while others do not. It is intriguing to speculate that differences in strain virulence or production of ARMs may be responsible for these differential PCD effects. Future studies will help to clarify this. A greater understanding of strain differences could also provide the framework for defining the influence of PCD on immunity in the overall disease process, because more virulent bacterial strains cause more severe disease.

Once these aspects of the host-pathogen interaction are defined in more detail, the implications of PCD on immunity can be addressed. Against the background of these variables, the emerging underlying theme of bacteria-modulated PCD is that induction or prevention of PCD can benefit either the host or the pathogen, depending on the factors discussed herein. In the broader context, the *in vivo* relevance of most findings to date remain unclear, because the vast majority of studies have not addressed the fundamental and more difficult question of the role of these processes in development of disease and immunity *in vivo*.

A BACTERIAL CALL TO ARMS: NOVEL TARGETS FOR THERAPY?

Because bacterial modulation of PCD may alter immunity, bacterial ARMs and their actions on apoptosis may represent novel therapeutic targets [407]. Indeed, it is clear that, at least experimentally, pathogen-induced PCD can be manipulated by pharmacological intervention to alter the outcome of infection. During pneumococcal infection, for example, inhibition of caspase activation brought about by pneumolysin and H_2O_2 reduces neuropathology in an animal model of pneumococcal meningitis [276]. On the other hand, prevention of PCD in alveolar macrophages in a murine model of lower respiratory tract infection impeded immune responses, thereby benefiting the microbe [273]. In listeriosis, the importance of suppressing FASL expression by T cells during early infection to prevent premature termination of the cell-mediated immune response has been emphasized [319], suggesting a possible immunomodulatory target. Inhibiting TRAIL in listeriosis appears to augment host immune responses, indicating another potential therapeutic strategy [408]. "Blanket" prevention of apoptosis, however, may ultimately abrogate protective immunity, because some aspects of the PCD response in listeriosis are important for immunity to infection [319]. In anthrax, antecedent exposure to sublethal doses of the lethal toxin may desensitize macrophages to toxin-triggered PCD; this process may be amenable to interventions designed to reduce the severity of anthrax infection [305]. Heat shock protein 72 [142] and epidermal growth factor [409,410] can protect against *C. difficile* toxin-A induced cytotoxicity through stabilizing actin, protecting mitochondria, and preventing caspase-9 activation. Overexpression of Rho proteins

can also limit *C. difficile* toxin B-induced cell death [411]. In animal models, deliberate colonization with *Saccharomyces boulardii* is an appealing probiotic approach to prevent *C. difficile* diarrhea. Yeast protease degrades bacterial toxin components, which reduces tissue damage [412,413]. Passive immunization with IgA to neutralize *C. difficile* toxic activity represents another therapeutic avenue [414]. Collectively, these examples illustrate that any therapeutic approach needs to be based on a sound understanding of the role of PCD in the overall disease process.

Studies have demonstrated that apoptosis activities in some cell types (e.g., T cells) can promote the intracellular growth of microbes in other cell types (e.g., macrophages) by impairing antimicrobial function [415,416]. More finely targeted therapeutic schemes directed at bacterial toxigenic activity related to PCD represent a logical avenue for further investigation. Future research detailing the molecular events that occur in particular disease states and ultimately alter PCD pathways will pave the way for a better understanding of possible interventional strategies.

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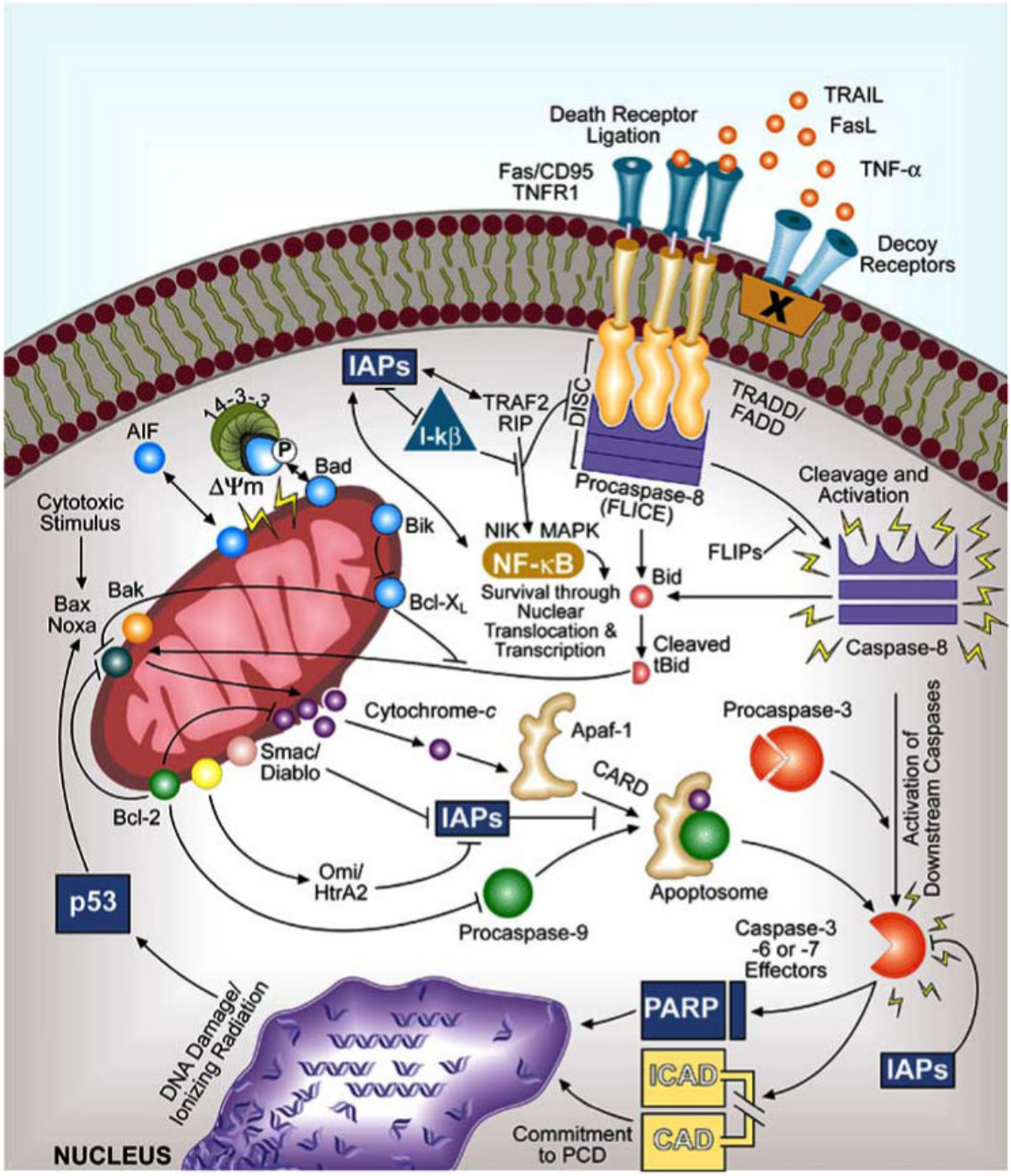

Fig. (1).

Mechanisms of intrinsic and extrinsic programmed cell death (PCD) in eukaryotic cells. Shown on the left is the current paradigm for intrinsic PCD, a pathway characterized by mitochondrial dysfunction, loss of mitochondrial membrane integrity (potential), and integrated signal transduction through various BCL-2 family members. Numerous BCL-2 proteins reside in the inner mitochondrial membrane space and contribute to cytochrome *c* release, subsequent caspase-9 activation, apoptosome formation, procaspase-3 cleavage, and terminal PCD events. As shown on the upper right, membrane-displayed death receptors relay initial receptor ligation by apoptotic ligands that provoke extrinsic PCD through death-effector domain-containing intracellular adaptor molecules TRADD and FADD with their corresponding procaspase

protein-docking regions; these regions act as signal transducers for initiator caspase-8 or -10 activation. Subsequent downstream effector caspase function results from cleavage of procaspase-3, -6, or -7 and then acts on suicide effectors such as PARP, which causes DNA breakdown and PCD. Various activators, inhibitors, and decoys regulate both intrinsic and extrinsic pathways; these regulators keep PCD pathways in check at various levels of early-, intermediate-, and late-stage events. Induction of the nuclear transcription factor NF-κB by its inducing kinase NIK (or MAPK) activates antiapoptotic gene transcription and regulates death pathways through factors such as IAPs and FLIPs. In this manner, NF-κB exerts prosurvival effects [189]. Normally, NF-κB activity is suppressed by I-κB, which is also regulated through interactions with IAPs.

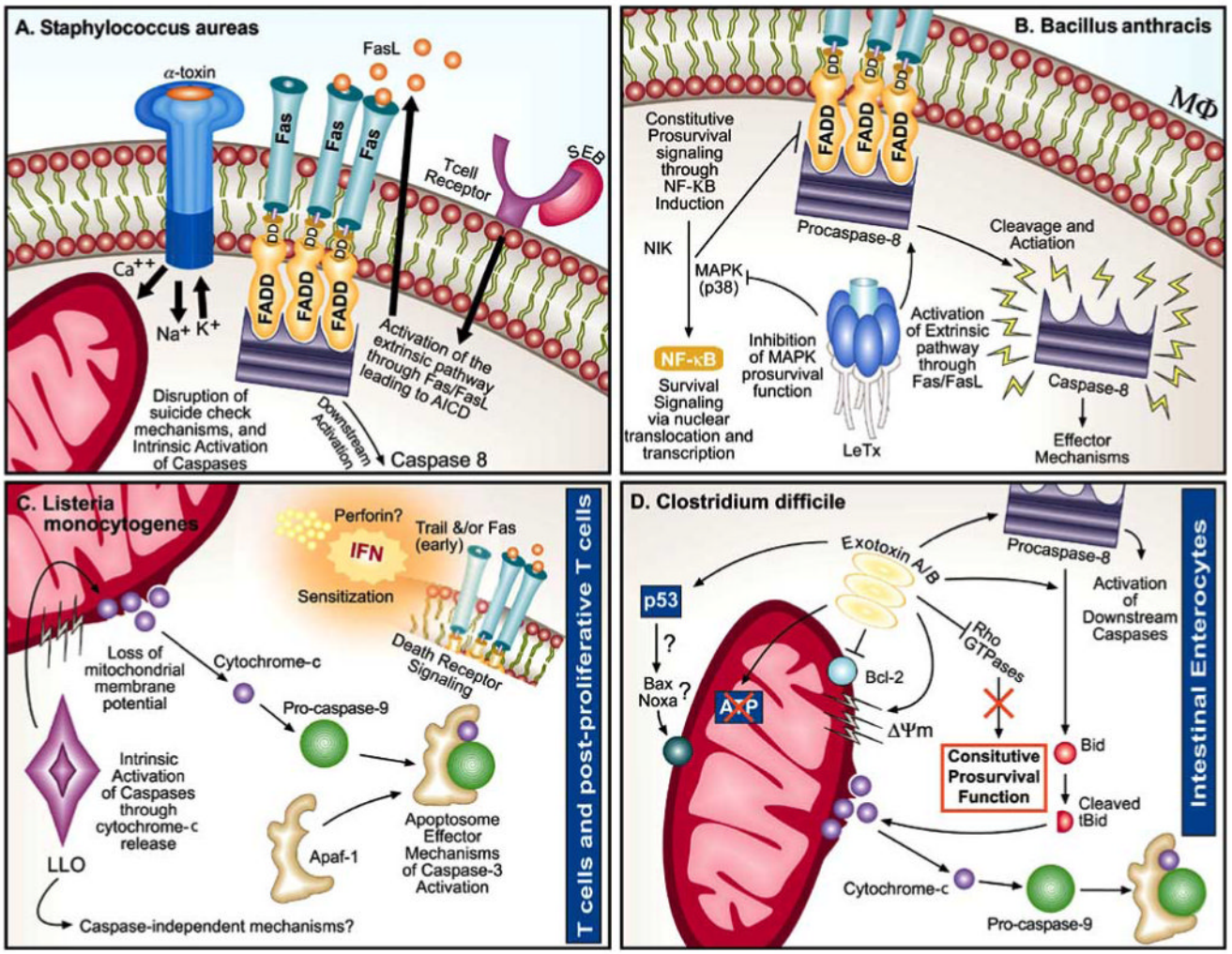

Fig. (2).

Programmed cell death (PCD) regulatory activities of gram-positive bacterial apoptosis regulatory molecules (ARMs). Numerous bacterial ARMs commandeer different elements of the intrinsic and extrinsic PCD pathways to seize control of the apoptotic machinery of the host cell; an activity that may improve the microbe's survival and persistence. In *S. aureus* infection (A), α-toxin causes pores to form in the host cell membrane, which disrupts ionic balance, and alters steady state intrinsic mitochondrial PCD activity. Additionally, SEB triggers activation-induced cell death in T cells by a mechanism centered on extrinsic PCD factors involving FAS-FASL signaling. LeTx produced by *B. anthracis* (B) inhibits MAPK activity in macrophages and triggers PCD by a mechanism that involves activation of caspase-8. PCD in activated T cells in response to *L. monocytogenes* listeriolysin O (C) is characterized by a variety of alterations in both the intrinsic and extrinsic PCD pathways. Perforin and IFN-γ appear to contribute to these alterations. Finally, PCD in intestinal enterocytes triggered by *C. difficile* exotoxin (D) is executed by inactivation of Rho GTPases and involves toxin-induced alterations in both the intrinsic and extrinsic PCD pathways.

Table 1

Apoptosis Regulatory Molecules of Gram-Positive Bacteria and their Cellular Targets

Bacteria & Disease Presentations	ARMs*	Identified Target Cell Type*	Reference
Staphylococcus aureus (skin and soft tissue, infections of bones and joints, respiratory tract infections, endovascular, gastrointestinal systems, sepsis)	SEB TSST-1 α-toxin SpA SEA, D, E ?†	$CD4^+$, T, B, Epi, Endo, Eosin B, Mono T, Mono, Endo B T keratinocytes, Osteo, Chondr	[37-75] [76,77] [49,78-81] [82,83] [84-86] [87-90]
Streptococcus agalactiae (pneumonia, meningitis, septic arthritis, osteomyelitis, sepsis)	β-hemolysin GRF ?	MØ, Hep MØ neurons, Mono	[28,29,91] [92] [93-96]
Streptococcus pneumoniae (otitis media, pneumonia, sinusitis, meningitis, sepsis)	PLY H_2O_2 cell wall ?	MØ, neurons, microglia neurons, microglia Mono DCs, PMNLs, Epi, T	[97-99] [97] [100] [101-109]
Streptococcus pyogenes (pharyngitis-tonsillitis, STTS, necrotizing fasciitis, impetigo, erysipelas, scarlet fever, post infection autoimmune disease)	NADase SLO SpeB glycoprotein ?	Keratinocytes Keratinocytes MØ, Epi fibrosarcoma cells PMNLs	[110] [110] [111-113] [114] [115]
Bacillus anthracis (anthrax, edema, sepsis)	LeTx PA	MØ, Endo, PMNLs Mono	[25,26,116-119] [116,120-122]
Listeria monocytogenes (granulomatosis infantiseptica, gastroenteritis, meningitis)	LLO ?	T, B, DCs Hep, Epi, γδ, $CD4^+$, $CD8^+$, B, Neurons, NK cells	[27,123-126] [127-131] [132-134]
Clostridium difficile (diarrhea, colitis, megacolon)	exotoxin A exotoxin B	Col, Mast, T, Epi, Eosin, Hep Epi, Hep, fibroblasts, Endo	[135-143] [143-148]
Clostridium perfringens (diarrhea, enteritis, gangrene)	CPE	Epi	[149,150]

Abbreviations: ARMs, apoptosis regulatory molecules; SEB, staphylococcal enterotoxin B; $CD4^+$, $CD4^+$ T cells; T, T cells; B, B cells; Epi, epithelial cells; Endo, endothelial cells; Eosin, eosinophils; TSST-1, toxic shock syndrome toxin-1; Mono, monocytes; SpA, superantigenic protein A; SEA, staphylococcal enterotoxin A; SEE, staphylococcal enterotoxin E; Osteo, osteoblasts; Chondr, chondrocytes; MØ, macrophages; Hep, hepatocytes; GRF, glucose-regulated factor; PLY, pneumolysin; DCs, dendritic cells; PMNLs, polymorphonuclear leukocytes; NADase, nicotinamide adenine dinucleotide-glucohydrolase; SLO, streptolysin O; SpeB, streptococcal pyrogenic exotoxin B; LeTx, lethal toxin; PA, protective antigen; LLO, listeriolysin O; γδ, γδ T cells; $CD8^+$, $CD8^+$ T cells; NK, natural killer cells; Col, colonocytes; Mast, mast cells; CPE, *Clostridium perfringens* enterotoxin.

*PCD induced in most cell types; those cell types in which PCD is inhibited are underlined.

†Question mark denotes unidentified ARMs.