

## Apoptosis in Bacterial Pathogenesis

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Increasing numbers of bacterial pathogens have been identified as mediators of apoptosis in vitro (1, 2). These pathogens infect many different tissues and include gram-positive and -negative bacteria as well as *Mycobacteria*. In this review, we analyze the role of apoptosis in bacterial pathogenesis. At least three pathogenic strategies involve programmed cell death: activation of apoptosis to destroy cells, utilization of apoptosis to initiate inflammation (3), and inhibition of host cell apoptosis. In addition, host cell apoptosis might represent a host defense reaction (4).

### Apoptosis as a host cell deletion mechanism

Activation of apoptosis might be an energetically efficient way for a tiny bacterial "David" to attack the eukaryotic "Goliath." The most obvious scenario where killing host cells would be beneficial is the induction of apoptosis in professional phagocytes, like PMN and macrophages, since these are the most dangerous cells for a bacterium. Interestingly, the induction of PMN apoptosis by a bacterial pathogen has yet to be described clearly. In contrast, several pathogens are known to induce apoptosis in macrophages. Two pathogens which efficiently kill macrophages are *Shigella spp* and *Salmonella spp*. Curiously, however, both of these pathogens have other mechanisms to evade the cell's antimicrobial activity. *Shigella* escapes from the phagosome into the cytoplasm of the cell (5) and *Salmonella* creates a special endosomal compartment (6, 7). As will be discussed below, it is likely that in *Shigella* and *Salmonella* infections the induction of apoptosis not only serves to delete cells, but also to initiate inflammation.

Situations in which apoptosis is exclusively a weapon to destroy cells have not been demonstrated directly in vivo. Nevertheless, some examples are very suggestive. Bacteria that produce exotoxins, like *Corynebacterium diphtheriae* (8), *Pseudomonas spp* (8), *Actinobacillus actinomycetemcomitans* (9, 10), and *Bacillus anthracis* (11), may benefit from killing macrophages before they ingest and destroy the bacteria. The in-

duction of apoptosis by *Bordetella pertussis* adenylate cyclase hemolysin (12, 13), which is expressed early during the course of colonization (12), may allow *Bordetella* to survive in the initial stages of the infection. After the bacteria have successfully colonized the tissue, *Bordetella* ceases to produce this toxin. Another situation where host cell killing would benefit the bacteria is the induction of apoptosis of epithelial cells, thus allowing sloughing off of the infected epithelium. This would serve as a vehicle for clearance and bacterial dissemination as suggested by recent work by Pier et al. (14) in *P. aeruginosa* infections. *P. aeruginosa* can induce apoptosis by producing exotoxin A (8).

Specific induction of apoptosis in immune cells can have serious consequences, as clearly demonstrated by HIV infections (15). HIV kills CD4<sup>+</sup> T cells and disables the capacity of the immune system to respond to antigens. The deletion of immune effector cells, including T cells and dendritic cells, has also been shown to occur in bacterial infections. Superantigens bind specific V $\beta$  T cell receptors and initiate a signaling cascade that culminates in apoptosis. During staphylococcal infections (16) certain cells expressing T cell receptors of the V $\beta$ <sub>8</sub> family in the mouse and other V $\beta$  families in humans are deleted. It was shown recently that *Listeria monocytogenes* can induce apoptosis in a dendritic cell line (17). Deletion of these efficient antigen-presenting cells could have serious consequences in mounting an immune response. Further experiments with explanted dendritic cells, in vivo demonstration of dendritic cell ablation, as well as the lack of antigen presentation during *Listeria* infections will be required to confirm the role of dendritic cell apoptosis in listeriosis.

### Apoptosis as a trigger of inflammation

In *Shigella* infections, apoptosis is mediated by the specific activation of the eukaryotic host cell programmed cell death mediator interleukin 1 $\beta$  (IL-1) converting enzyme (ICE).<sup>1</sup> We propose the model that ICE mediated apoptosis in macrophages allows the efficient release of IL-1 $\beta$  which triggers the acute inflammation typical of shigellosis (3). Shigellosis, or bacillary dysentery, is an acute diarrheal disease that is characterized by the presence of blood and mucus in the stools. Upregulation of apoptosis has been shown in both animal models of shigellosis (18) and in dysenteric patients (19).

IL-1 $\beta$  is a major proinflammatory cytokine produced mainly by macrophages. It is synthesized as a biologically inactive precursor that lacks a secretion signal sequence and accumulates in the cell cytoplasm (20). The 31-kD IL-1 $\beta$  pre-

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1. Abbreviations used in this paper: ICE, IL-1 $\beta$  converting enzyme; Ipa, invasion plasmid antigen.

cursor is specifically cleaved by ICE to a 17-kD mature form. ICE is a cysteine protease of the caspase family. Caspases include at least 12 different human enzymes and are conserved among phylogenetically distant animals, from worms and flies to humans. Caspases have different substrate specificity but can induce apoptosis when overexpressed in tissue culture cells. ICE (caspase 1) is the only member of the family that efficiently cleaves the proinflammatory cytokine IL-1 $\beta$ . All caspases are translated as zymogens that can autocleave themselves. Healthy growing cells produce large amounts of several caspases but do not spontaneously undergo apoptosis. This suggests that caspase activation, and probably access to relevant substrates, must be tightly regulated within the context of the cell. The ubiquitous production of caspases also indicates that cells are poised to undergo apoptosis at any point. How cells keep their caspases in check is still very poorly understood (21–23).

Bacteria have evolved molecules that deregulate caspases to induce apoptosis. *Shigella flexneri* encodes an invasin, invasion plasmid antigen (Ipa)B, that binds to ICE (24). IpaB either activates the cleavage of the ICE precursor or makes the mature enzyme more efficient. IpaB is exquisitely specific for ICE. We have shown recently that macrophages isolated from ICE knockout mice are insensitive to *Shigella* cytotoxicity (Sansonetti, P.J., J. Arondel, A. Phalipon, S. Banerjee, K. Thirumalai, and A. Zychlinsky, manuscript in preparation). This was an intriguing result because macrophages from ICE  $-/-$  mice die normally after other apoptotic stimuli (26).

Concomitant with apoptosis, macrophages infected with *S. flexneri* release mature IL-1 $\beta$  (27). Furthermore, pretreatment of experimental animals with IL-1 receptor antagonist before a challenge with *Shigella* prevents inflammation, indicating that IL-1 is crucial to the establishment of disease (28). ICE  $-/-$  mice infected with *S. flexneri* are unable to mount the acute inflammatory reaction that characterizes this infection (Sansonetti, P.J., J. Arondel, A. Phalipon, S. Banerjee, K. Thirumalai, and A. Zychlinsky, manuscript in preparation). Taken together, these data suggest that, within the context of *Shigella* pathogenesis, ICE activation serves the dual purposes of cleaving IL-1 $\beta$  and initiating apoptosis.

It remains to be established how IL-1 $\beta$  is released from the cytoplasm of the apoptotic macrophage. This is particularly important since it has been demonstrated clearly that early on in the apoptotic process cells express, on their surface, a variety of molecules that allow phagocytes to detect them (29). Recognition of apoptotic cells by phagocytes is crucial to avoid spillage of intracellular contents and the consequent immunological recognition during developmental cell death. Whether macrophage apoptosis allows intracellular contents to leak, or whether IL-1 $\beta$  is specifically secreted during apoptosis is not known.

Infections with two other pathogens suggest that ICE-dependent apoptosis is relevant in other bacterial diseases. *Salmonella* can also induce apoptosis in macrophages (30–32). This bacterium encodes an invasin, *Salmonella* invasion protein (Sip)B, which has homology to IpaB (33–35) and is a likely candidate to be an ICE-activating protein. Differences between shigellosis and salmonellosis suggest that inflammation is initiated with a different time course during pathogenesis. How IL-1 $\beta$  is regulated during salmonellosis is still an open question. Anthrax toxin lethality has been shown to be dependent on macrophages and upon IL-1 release (11), although

there is no direct evidence of ICE activation or macrophage apoptosis so far. Future research will explore whether ICE-mediated apoptosis is a proinflammatory event in diseases mediated by nonbacterial pathogens.

Recently, Rogers et al. (36) identified apoptotic cells as a source of proinflammatory cytokines in *Listeria* infections. In this report, *Listeria* was shown to induce apoptosis in hepatocytes of infected animals. Apoptotic cells released a PMN chemotactic factor which remains to be characterized.

#### *Inhibition of apoptosis*

Several pathogens, including herpes-, pox-, and baculoviridae have evolved or acquired genes that specifically inhibit the apoptotic machinery (37). Parasitic pathogens like *Leishmania donovani* seem to also inhibit apoptosis (38). Since *Leishmania* amastigotes are strictly intracellular, inhibition of host cell death could be advantageous for the parasite.

Although inhibition of host cell apoptosis would be beneficial for intracellular pathogens, this mechanism has not yet been described for bacterial infections. Nevertheless, bacterial products, like endotoxin, have been shown to delay apoptosis in PMN (39) and macrophages (40), although the significance in pathogenesis is not understood.

#### *Apoptosis as a defense mechanism*

*Mycobacteria* reside in a unique vacuolar environment within the macrophage wherein the bacteria proliferate untouched by the host microbicidal agents (41). How the host destroys *Mycobacteria* is a subject of intense research. Molloy et al. (4), showed that when macrophages infected with *Bacillus Calmette-Guérin* are stimulated to undergo apoptosis, there is a great reduction in the number of surviving bacteria compared with toxic mechanisms that do not activate apoptosis. Interestingly, activation of the Fas receptor, a different apoptotic stimulus, in *M. avium-M. intracellulare* infected macrophages did not result in bacterial killing (42). It remains to be determined whether these data indicate that the bactericidal potential of apoptotic macrophages depends on the apoptotic pathway initiated in the host cell or on the species of *Mycobacteria*.

It will be important to determine whether the bactericidal factors released by apoptotic cells are distinct from those in lysosomes. Alternatively, the antibacterial effect might be the result of the in vitro exposure of the *Mycobacteria* to cellular components that the bacteria is shielded from in the intracellular environment. Whether apoptosis can be bactericidal in infections with other bacteria has not been studied. Nevertheless, the fate of the bacteria is crucial in the two mechanisms described above: cell ablation and initiation of inflammation. If bacteria are killed through the apoptotic process, it would only be an effective weapon for extracellular bacteria.

The bactericidal role of apoptosis in vivo will determine whether this is in fact a host defense mechanism. Extensive apoptosis has been shown in caseating granulomas in lungs of tuberculosis patients (43). Whether this apoptosis decreases the bacterial load is unknown. *Mycobacteria* can also induce apoptosis in macrophages through TNF- $\alpha$  (43, 44). The intricate balance between the bactericidal effect of apoptotic macrophages and the induction of apoptosis by *Mycobacteria* remains to be understood.

Future research will undoubtedly focus on the relevance of bacterial-induced apoptosis in pathogenesis. The study of bacterial-induced apoptosis will not only provide new insights into

the apoptotic pathway, but also will uncover new therapeutic avenues to manage and eradicate bacterial diseases.

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