Macrophage apoptosis in microbial infections

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SUMMARY

Upon infection with a pathogen, eukaryotic cells can undergo programmed cell death as an ultimate response. Therefore, modulation of apoptosis is often a prerequisite to establish a host-pathogen relationship. Some pathogens kill macrophages by inducing apoptosis and thus overcome the microbicidal arsenal of the phagocyte. Apoptotic macrophages, on the other hand, can elicit an inflammation by secretion of proinflammatory cytokines. *Shigella flexneri*, the aetiological agent of bacillary dysentery, induces apoptosis in macrophages which, in agony, specifically release mature interleukin-1β (IL-1β). This cytokine attracts neutrophils (PMN) to the site of infection resulting in the massive colonic inflammation characteristic of bacillary dysentery. Shigellosis represents a paradigm of a proinflammatory apoptosis in a bacterial infection. The molecular link between apoptosis and inflammation is interleukin-1β converting enzyme (ICE) which is activated during macrophage apoptosis and binds to IpaB, a secreted *Shigella* protein.

Key words: Bacteria, pathogenicity, macrophages, apoptosis, inflammation, parasites.

PROGRAMMED CELL DEATH IN DISEASES

Normal development of multicellular organisms requires death and disposal of certain individual cells for the benefit of the whole organism. Surplus, damaged or undesirable cells commit suicide by enacting an evolutionarily conserved, intrinsic programme referred to as apoptosis or programmed cell death (Ameisen, 1996; Arends & Wyllie, 1991; Steller, 1995). Apoptotic cells are characterized by a distinct morphology and, on a biochemical level, by the cleavage of nuclear DNA at the internucleosomal boundaries to fragments of about 200 bp. Apoptosis not only takes place in structural processes like morphogenesis, metamorphosis or tissue homeostasis (Raff, 1992), but it is also involved in eliminating potentially harmful cells such as autoreactive thymocytes and senescent neutrophils (PMN) (Ellis, Yuan & Horvitz, 1991). Macrophages recognize and phagocytose apoptotic cells and thus clear the debris of the dying cells from the tissue. In summary, the very purpose of canonical programmed cell death is the noninflammatory removal of bothersome cells.

Many diseases are associated with an imbalance of apoptosis resulting either in a detrimental increase or in depletion of certain cells. In malignancies, autoimmune disorders and viral infections apoptosis is blocked, whereas in neurodegenerative disorders or AIDS increased apoptosis occurs (Thompson, 1995). During viral infections, programmed cell death is employed as a defense strategy. Virally infected cells are prone to die by apoptosis triggered either by effector cells of the immune system or endogenously. It is, therefore, not surprising that a number of viruses are capable of suppressing apoptosis of their host cells, thus sustaining an intracellular infection. Interestingly, some viral anti-apoptotic proteins directly interfere with the components of the cell death machinery. CrmA of cowpox virus and p35 of baculovirus inhibit ICE-like proteases which are crucial components of the apoptotic programme. Inhibition of ICE not only prevents apoptosis but also the production of mature IL-1β, a potent proinflammatory cytokine which, upon release by infected cells, can alert the immune system (Vaux, Haecker & Strasser, 1994). Other viral proteins like BHRF1 of Epstein–Barr virus are homologous to Bcl-2, an inhibitor of apoptosis, or they inactivate p53 which mediates the apoptotic response to DNA damage (Thompson, 1995).

Intracellular pathogens can also cause apoptosis of their host cells. For example, human immunodeficiency virus (HIV) infects and kills CD4$^+$ lymphocytes. Specific depletion of these cells accounts for immunodeficiency and might also aid in the establishment of a chronic infection (Barr & Tomei, 1994). Another example is *Shigella flexneri*, an intracellular bacterium that kills macrophages by inducing apoptosis and thereby overcomes the bactericidal potential of this host cell. The dying macrophages secrete IL-1β which triggers an acute inflammation of the colonic mucosa. Shigellosis is a self-limiting disease, probably because the macrophage's apoptosis is not an immunologically silent, altruistic suicide, but rather a proinflammatory process eventually leading to clearance of the infection (Zychlinsky & Sansonetti, 1997).

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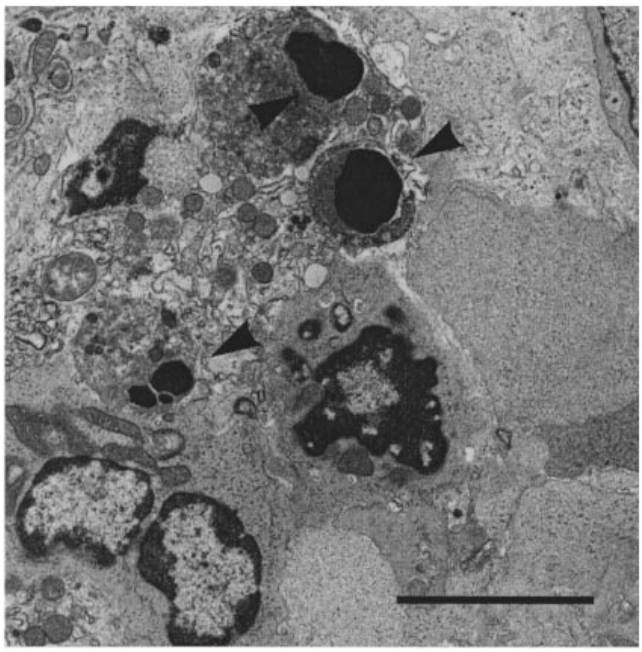


Fig. 1. Electronmicrograph of apoptotic cells from a lymphoid follicle infected with *Shigella flexneri*. Arrowheads point to apoptotic nuclei. Bar represents 5 μm.

After an overview of the events involved in Shigella-directed phagocytosis we will focus in this review on macrophage apoptosis induced by *Shigella* and other pathogens. We will summarize the evidence that in shigellosis macrophage apoptosis is a proinflammatory event and, in light of the concept that pathogen-induced apoptosis might provoke an inflammation, we will also review recent findings on the pathogenesis of other infections.

PATHOGENESIS OF SHIGELLOSIS

The enterobacterium *Shigella* is the aetiological agent of bacillary dysentery, a severe form of diarrhoea characterized by blood and mucus in the stool. Typical symptoms are painful abdominal cramps, nausea and fever. The pathology of the disease is due to colonic epithelial cell destruction and massive inflammation (reviewed by Siebers & Finlay, 1995; Zychlinsky *et al*. 1994*c*). Dysentery is self-limiting in healthy individuals; however, because of dehydration, shigellosis can be a life-threatening disease and is frequently fatal in children.

The pathogenicity of *Shigella* is associated with its ability to invade colonic cells which it can enter only from the basolateral side but not from the apical side (Mounier *et al*. 1992). As a consequence, *Shigella* relies on M-cells, specialized antigen sampling cells of the mucosa-associated lymphoid tissue (MALT), to penetrate the epithelial barrier (Perdomo *et al*. 1994*b*; Wassef, Keren & Mailloux, 1989). After passage through M cells, associated resident tissue macrophages readily phagocytose the pathogen. Inside the macrophages, *Shigella* escapes the phagolysosome, and kills its host cells by inducing apoptosis (Zychlinsky, Prévost & Sansonetti, 1992). Fig. 1 shows apoptotic cells in a lymphoid follicle infected with *S. flexneri*.

The dying macrophages secrete IL-1 which initiates inflammation (Zychlinsky *et al*. 1996). PMN are chemoattracted to the site of infection and destroy the integrity of the epithelium as they traverse into the lumen of the colon (Perdomo *et al*. 1994*b*). Transmigration of PMN facilitates mucosal access for the bacteria by opening intercellular junctions. The current model of inflammation-enhanced shigellosis is shown in Fig. 2. This model is substantiated by the following findings: (1) a monoclonal antibody against $\alpha_M\beta_2$ integrin (CD18) which neutralizes PMN adhesion to epithelial cells, largely reduced infection in the rabbit ligated ileal loop model (Perdomo *et al*. 1994*b*) and blocks PMN transmigration *in vitro* (Perdomo *et al*. 1994*b*); (2) intravenous infusion of IL-1 receptor antagonist reduces inflammation and decreases the number of bacteria in mucosal tissue of rabbit ileal loops (Sansonetti *et al*. 1995); and (3) disruption of tight cell junctions by treatment with EGTA greatly enhances cell infection of a polarized colonic cell monolayer with *Shigella* via the basolateral route (Mounier *et al*. 1992).

BOTH SHIGELLA-INDUCED PHAGOCYTOSIS AND INTRACELLULAR MOVEMENT EMPLOY THE ACTIN CYTOSKELETON OF EPITHELIAL CELLS

Epithelial cells of the colonic mucosa internalize *Shigella* by a process resembling macropinocytosis. This pathogen-directed phagocytosis is characterized by membrane ruffling, polymerization of actin filaments and actin bundling by plastin (reviewed by Ménard, Dehio & Sansonetti, 1996*a*). Concomitant with bacterial entry, a number of host cell signalling events take place, including cortactin tyrosine phosphorylation and recruitment of small Rho GTPases (Ménard *et al*. 1996*a*).

Bacteria-induced uptake of *Shigella* is a complex process involving more than 30 gene products (Ménard *et al*. 1996*a*). The genes conferring entry into epithelial cells are located on a 31 kb fragment of the 220 kb virulence plasmid characteristic of all virulent *Shigella* strains. The gene products include IpaA, IpaB, IpaC and IpaD (*i*nvasion *p*lasmid *a*ntigen) and the chaperone IpgC. The Ipa proteins are indispensable for entry into epithelial cells and lysis of the phagocytic vacuole (High *et al*. 1992; Ménard, Sansonetti & Parsot, 1993). A type III secretion apparatus, encoded by the *Mxi* and *Spa* operons, secretes the Ipa proteins (Ménard *et al*. 1996*a*) and is controlled by IpaB and IpaD (Ménard, Sansonetti & Parsot, 1994*a*).

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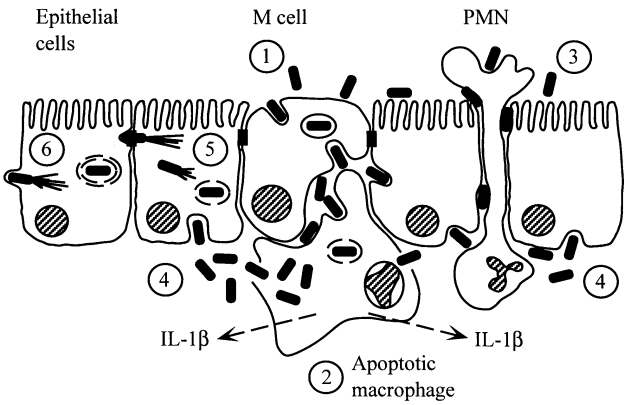


Fig. 2. Passage of *Shigella* from the colonic lumen to the mucosa. (1) Shigellae enter the intestinal epithelium via M cells; (2) resident macrophages phagocytose the bacteria and are killed by apoptosis. The dying macrophages release mature IL-1β which initiates inflammation; (3) chemoattracted PMN destroy the integrity of the epithelial barrier; (4) *Shigella* invades enterocytes basolaterally through bacteria-induced phagocytosis; (5) endocytosed bacteria escape the vacuole, move in the cytoplasm by actin polymerization, spread to neighbouring cells *via* cell junctions; and (6) lyse the double membrane of the protrusions to continue the intraepithelial cell-to-cell spread.

Presynthesized IpaB and IpaC are separately stored in the bacterial cytoplasm, each in transient association with the chaperone IpgC (*i*nvasion *p*lasmid *g*ene) which prevents the degradation of IpaB (Ménard *et al*. 1994*b*). Upon contact with a target cell or components of the extracellular matrix, a soluble IpaB–IpaC complex is rapidly released (Ménard *et al*. 1994*a*; Watarai *et al*. 1995). Latex beads coupled to immunopurified IpaB-IpaC complexes are efficiently internalized into HeLa cells thus providing that this complex is sufficient to promote uptake. Interestingly, these beads elicit membrane projections and actin polymerization as seen during wild type *Shigella* invasion (Ménard *et al*. 1996*b*).

After entry and lysis of the phagosome, *Shigella* moves intracellularly and spreads to adjacent epithelial cells by exploiting components of the host cell cytoskeleton. IcsA (*i*ntra/inter*c*ellular *s*pread) mediates the directional polymerization of actin filaments (Bernardini *et al*. 1989). Actin-based motility contributes largely to the virulence of *Shigella*, since strains lacking *icsA* only form small localized inflammatory lesions in lymphoid follicles but do not cause dysentery (Sansonetti *et al*. 1991). IcsA contains a consensus sequence for cAMP-dependent kinases which upon phosphorylation by host cell kinases limits the intracellular spread of *Shigella* (d'Hauteville & Sansonetti, 1992). This phosphorylation consensus site is also the cleavage site for proteolytic processing of IcsA which is a prerequisite for its unipolar localization in the outer membrane (d'Hauteville *et al*. 1996).

The fibres formed by IcsA-dependent actin polymerization propel the bacteria through the cell and eventually deliver it to adjacent cells (Goldberg & Theriot, 1995). Cell to cell spread is dependent on cadherins, a family of calcium-dependent cell adhesion molecules that are components of intermediate junctions (Sansonetti *et al*. 1994). Finally, the endocytosed protrusions, consisting of two membranes, are lysed by IcsB (Allaoui *et al*. 1992), and the invasion may continue.

SHIGELLA KILLS MACROPHAGES BY APOPTOSIS

Shigella flexneri does not sustain an infection within macrophages but rapidly after infection escapes the phagolysosome and kills its host cells by apoptosis. Dying macrophages infected either with a virulent laboratory strain of *S. flexneri* (Zychlinsky *et al*. 1992) or with clinical *Shigella* isolates (Guichon & Zychlinsky, 1997) exhibit the distinct characteristics of apoptotic morphology: blebbing of the cytoplasmic membrane, vacuolization of the cytoplasm yet overall maintenance of organelle structure and chromatin condensation at the nuclear boundary. Conversly, HeLa cells infected with *S. flexneri* stay viable and intact for at least 12 h and show only signs of metabolic stress but not the ultrastructural hallmarks of programmed cell death (Mantis, Prévost & Sansonetti, 1996).

Induction of apoptosis by virulent *Shigella* is significant *in vivo*. After infection of rabbit Peyer's patches, apoptotic cells were labelled by the TUNEL technique (*t*erminal d-transferase-mediated d*U*TP-biotin *n*ick *e*nd *l*abelling) and quantified. In this assay, wild type *Shigella* caused apoptosis in cells of the lympoid tissue, in contrast to a plasmidless, nonvirulent strain and an avirulent strain capable of colonizing Peyer's patches. Apoptotic cells were identified by immunofluorescence as macrophages, B and T cells (Zychlinsky *et al*. 1996).

APOPTOTIC MACROPHAGES RELEASE MATURE IL-1β, A PROINFLAMMATORY CYTOKINE

Macrophages are among the most effective cells in signalling inflammation by secreting proinflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor α (TNFα) (Dinarello, 1992). IL-1, in contrast to IL-6 and TNFα, is not secreted by activated macrophages but rather stored in the cytoplasm. IL-1α and IL-1β, the two forms of IL-1, bind to the same receptor and have very similar biological activity including chemotactic attraction of PMN and macrophages. IL-1α and IL-1β lack classical signal sequences and are cleaved by either calpain, a calcium-dependent protease, or by ICE, the interleukin-1β converting enzyme, respectively (Dinarello & Margolis, 1995). Whereas IL-1α is biologically active as precursor and mature protein,

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IL-1β is active only after cleavage of the 31 kDa proprotein to the 17·5 kDa mature form by ICE. Hence, ICE is a pivotal immunomodulatory enzyme.

Processing and secretion of IL-1 by macrophages depends on the nature of injury imposed (Hogquist *et al*. 1991). Only during apoptotic death, but not as a consequence of necrosis, is pro-IL-1β processed and mature IL-1β secreted. Mature IL-1β, therefore, might be a marker cytokine for apoptotic macrophages.

Upon *in vitro* infection of stimulated peritoneal macrophages, *S. flexneri* was shown to elicit specifically the rapid secretion of both preformed precursor IL-1α and mature IL-1β. Stimulation of IL-1β secretion was restricted to the wild type *S. flexneri* strain and neither a noninvasive derivative nor a noncytotoxic yet invasive control strain provoked the release of mature cytokine. Conversly, IL-6 and TNFα were not detected, since the macrophages were killed by *Shigella* before *de novo* biosynthesis could take place (Zychlinsky *et al*. 1994*a*; 1996).

To substantiate the role of IL-1 in the pathogenesis of *Shigella*, rabbit ileal loops were infected with the pathogen after intravenous administration of IL-1 receptor antagonist (IL-1ra). IL-1ra is a macrophage product which inhibits IL-1 competitively binding to the IL-1 receptor. Continuous infusion of the animals with recombinant IL-1ra reduced inflammation, destruction of mucosal tissue and bacterial invasion thus proving the crucial role of IL-1 in the pathogenesis of shigellosis (Sansonetti *et al*. 1995).

In summary, there is convincing evidence that *Shigella*-infected apoptotic macrophages secrete mature IL-1β which initiates inflammation. IL-1, in turn, boosts the secretion of other proinflammatory cytokines leading to a full-fledged inflammation. The orchestrated upregulation of proinflammatory cytokines in the colon chemoattracts PMN which destroy the epithelium and in shigellosis finally clear the infection. Thus, in *Shigella* pathogenesis the apoptotic release of IL-1β is a proinflammatory event.

IPAB BINDS ICE, THE ENZYME LINKING APOPTOSIS AND INFLAMMATION

Only recently the molecular link of *Shigella*-induced macrophage apoptosis and the onset of inflammation was unravelled. *Shigella* has to reach the cytoplasm of macrophages to induce apoptosis. To show which protein is necessary for apoptosis, the requirement of Ipa proteins for lysis of the phagosome had to be bypassed. This was accomplished by constructing non-polar mutant *Shigella* strains that were deficient in single Ipa proteins but secreted low amounts of *E. coli* hemolysin. With this system IpaB, a 62 kDa protein, but not IpaC or IpaD, was shown to be necessary to induce apoptosis (Zychlinsky *et al*. 1994*b*). Microinjecting purified IpaB into peritoneal macrophages caused these cells to undergo apoptosis thus proving that IpaB is sufficient to elicit programmed cell death (Chen *et al*. 1996*b*).

Macrophage target proteins of IpaB were identified by using purified IpaB as a ligand in affinity chromatography. Four proteins of 45, 33, 20 and 10 kDa, respectively, were specifically retained by an IpaB affinity column and were identified as precursors and mature fragments of ICE or a closely related protein. Furthermore, inhibition of ICE with the peptide inhibitor acetyl-YVAD-CHO abrogated *Shigella*-induced macrophage apoptosis as well as release of mature IL-1β (Chen *et al*. 1996*b*). Hence, in macrophage apoptosis induced by *Shigella* the proapoptotic and proinflammatory potential of ICE converges (Fig. 3).

ICE originally was identified as a novel cysteine protease that processes pro-IL-1β to its mature, proinflammatory form (Cerretti *et al*. 1992; Thornberry *et al*. 1992). Subsequently, it was found that the cell death gene *ced-3* of the nematode *Caenorhabditis elegans* is homologous to ICE (Yuan *et al*. 1993) and that overexpression of either cysteine protease in fibroblasts results in programmed cell death (Miura *et al*. 1993). These findings suggest that mediation of programmed cell death by cysteine proteases is evolutionarily conserved. ICE is synthesized as a 45 kDa proenzyme, which after heterologous cleavage or autoprocessing at aspartate sites releases a 11 kDa N-terminal prodomain and an internal 2 kDa fragment to form the active $(p20)_2$/$(p10)_2$ heterotetramer (Whyte, 1996).

To date, 10 homologues of the still growing family of ICE/CED-3 cysteine proteases have been identified and subdivided into three groups based on their degree of similarity (ICE-, CPP32- and ICH-1-like proteases). For these related proteases the term 'caspase' ('*c*ysteine protease cleaving after *asp*artic acid') has been suggested (Alnemri *et al*. 1996). Interestingly, ICE is largely redundant for apoptosis but indispensable for processing of IL-1β since mutant mice deficient in ICE appear healthy but require a larger dose of endotoxin to induce toxic shock (Kuida *et al*. 1995; Li *et al*. 1995). CPP32-deficient mice, on the other hand, suffer from decreased apoptosis in the brain and die prematurely (Kuida *et al*. 1996).

MACROPHAGE APOPTOSIS IN OTHER BACTERIAL INFECTIONS

Induction of apoptosis by bacterial pathogens is widely used as a cytotoxic strategy among both extra- and intracellular bacteria (reviewed by Chen & Zychlinsky, 1994). Apart from IpaB mentioned above, the apoptotic arsenal comprises (1) A-B toxins which inhibit protein synthesis, (2) enzymes that generate second messengers like cAMP, (3) strepto-

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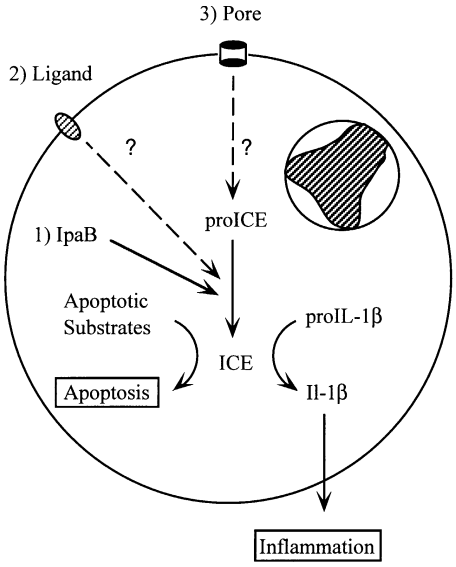


Fig. 3. Activation of the bi-functional enzyme ICE in macrophages results in convergence of apoptosis and inflammation. Macrophage apoptosis can be induced by (1) factors directly delivered to the cytoplasm like IpaB; (2) transmembrane signalling events involving, e.g., extracellular ATP, or (3) poreforming proteins.

and staphylococcocal superantigens that induce apoptosis by crosslinking certain T-cell receptors and (4) pore-forming proteins like *Staphylococcus aureus* α-toxin or hemolysin of enteropathogenic *Escherichia coli*. The variety of mechanistically different apoptogenic toxins reflects the plethora of pathways leading to programmed cell death.

A subset of bacteria or microbial products cause apoptosis in macrophages thus killing the phagocyte by exploiting its intrinsic cell death programme (Table 1). These pathogens very efficiently overcome the microbicidal activities of the phagocyte by expression of comparably small amounts of toxin. However, since macrophage apoptosis also bears the potential of a proinflammatory event, the initial success of the pathogen finally might contribute to its restriction and elimination. Host–pathogen relationships involving proinflammatory apoptosis may not be restricted to shigellosis but rather may apply to other diseases such as anthrax, diphtheria, whooping cough, periodontitis, listeriosis, salmonellosis and parasitic infections (Zychlinsky & Sansonetti, 1997).

Only recently it was found that both *Salmonella typhi* and *S. typhimurium* induce apoptosis in macrophages, although with slower kinetics than *Shigella* (Chen, Kaniga & Galan, 1996*a*; Lindgren, Stojilkovic & Heffron, 1996; Monack *et al*. 1996). Hence, *Salmonella* not only evades the host immune system by sustaining an intramacrophage infection but also by depletion of phagocytes. The ability to cause macrophage programmed cell death is restricted to invasive *S. typhimurium*; noninvasive mutant strains do not induce apoptosis (Monack *et al*. 1996). Interestingly, *Salmonella* spp. synthesize SipB, a protein homologous to IpaB from *Shigella*. This protein, like IpaB, is required for entry into epithelial cells (Kaniga *et al*. 1995) and is necessary to induce apoptosis (Chen *et al*. 1996*a*). This is rather surprising considering the different ways *Shigella* and *Salmonella* interact with phagocytic and nonphagocytic cells and the differences in the course of shigellosis and salmonellosis. Whereas the former disease manifests as an acute, self-limiting diarrhoea, localized to the colon, the latter can cause a severe chronic, systemic illness (typhoid fever). However, the phenotype of a particular infection depends on the complete pattern of the pathogen's virulence factors which likely are not identical for *Shigella* and *Salmonella*.

Additionally, IpaB and SipB are related to YopB (*Yersinia* outer protein), a *Yersinia*-secreted protein involved in translocation of Yop effector proteins across the target cell plasma membrane (Hakansson *et al*. 1993, 1996). YopB is also implicated in suppression of TNFα expression (Beuscher *et al*. 1995). IpaB, as outlined above, causes apoptosis in macrophages which is accompanied by the secretion of proinflammatory IL-1β. Hence, closely related bacterial virulence factors can act either as suppressor or as effector of inflammation depending on the pathogenic strategy of the infectious agent.

Listeria monocytogenes does not induce apoptosis in a macrophage cell line (Zychlinsky *et al*. 1992) but in hepatocytes (Rogers *et al*. 1996). The latter cells were shown to chemoattract PMN to the site of infection by an as yet unidentified factor. Consequently, the concept of apoptosis as a proinflammatory event might not only apply to macrophages releasing mature IL-1β but also to other apoptotic cells releasing different proinflammatory cytokines.

Lethal anthrax toxin is a putative zinc protease synthesized by *Bacillus anthracis*. At high concentrations, this toxin lyses macrophages *in vitro*. The systemic toxic shock, characteristic of anthrax, is specifically mediated by macrophages and can be counteracted *in vivo* by administering antiserum against IL-1 or IL-1 receptor antagonist (Hanna, Acosta & Collier, 1993). Since sublytic stimulation of macrophages with lethal anthrax toxin also provokes the secretion of IL-1, this toxin might be a mediator of apoptosis possibly by proteolytically activating ICE.

Inhibition of protein synthesis efficiently induces apoptosis in eukaryotic cells. Diphtheria toxin of *Corynebacterium diphtheriae* and exotoxin A of *Pseudomonas aeruginosa* mediate apoptosis by ADP-ribosylation of elongation factor-2 (Morimoto & Bonavida, 1992). These toxins and other inhibitors of protein synthesis induce apoptosis in a human monoblastoid cell line (Kochi & Collier, 1993) and, therefore, might also be cytotoxic for macrophages *in vivo*. Proinflammatory macrophage apoptosis, in turn, might contribute to the formation of a

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Table 1. Bacterial toxins inducing apoptosis in macrophages (for references see text)

Bacterium	Toxin	Mode of action
Shigella flexneri	IpaB	Binding of ICE
Salmonella typhi(murium)	SipB	?
Bacillus anthracis	Lethal antrax toxin	Proteolysis?
Corynebacterium diphtheriae	Diphteria toxin	Inhibition of protein synthesis
Pseudomonas aeruginosa	Exotoxin A	Inhibition of protein synthesis
Bordetella pertussis	Adenylate cyclase	Generation of cAMP
	Hemolysin	
Actinobacillus actinomycetemcomitans	Leukotoxin	Formation of pores Changes in Ca^{2+} concentration
Escherichia coli	Hemolysin	Formation of pores
Staphylococcus aureus	α-toxin	Formation of pores

diagnostically characteristic feature of diphtheria: the pharyngal pseudomembranes, consisting of fibrin, bacteria and inflammatory cells.

Bordetella pertussis, the causative agent of whooping cough, can enter and survive in epithelial cells and macrophages. In a monocyte/macrophage cell line *B. pertussis* causes apoptosis which indispensably involves the calmodulin-dependent adenylate cyclase hemolysin, a RTX toxin, but not pertussis toxin, an ADP-ribosylating A-B toxin (Khelef, Zychlinsky & Guiso, 1993). Murine alveolar macrophages also undergo apoptosis upon infection with *B. pertussis* indicating that promotion of cell death might contribute to bacterial survival and escape from the host immune response *in vivo*.

Pore-forming toxins can provoke either apoptosis or necrosis depending on their concentration. *Actinobacillus actinomycetemcomitans*, a causative agent of periodontitis, synthesizes a pore-forming leukotoxin which is another member of the bacterial RTX toxin family (reviewed by Czuprynski & Welch, 1995). This toxin is selectively cytotoxic for monocytes and PMN and kills T-lymphocytes by apoptosis and necrosis (Mangan *et al*. 1991). Leukotoxicity might be critical in establishing a chronic periodontal infection by *A. actinomycetemcomitans*. In sublytic concentrations, hemolysin of pathogenic *Escherichia coli* stimulates IL-1 release from monocytes *in vitro* (Bhakdi *et al*., 1990), thereby likely causing apoptosis in these cells. Both hemolysin and staphylococcal α-toxin (Bhakdi *et al*., 1990) induce internucleosomal DNA fragmentation and apoptosis in T-lymphocytes (reviewed by Chen & Zychlinsky, 1994) and thus probably contribute to immunosuppression by pathogenic *E. coli* and *S. aureus*.

APOPTOSIS IN PARASITIC DISEASES

Depending on their pathogenic strategy, protozoan parasites either downregulate or induce apoptosis of immune cells. Macrophages are the exclusive host cells of *Leishmania*. Visceral leishmaniasis (kala-azar) is caused by *L. donovani* which was shown to suppress macrophage apoptosis by induction of TNFα expression thereby sustaining an intracellular infection (Moore & Matlashewski, 1994). In contrast, *Entamoeba histolytica* causes contact-dependent apoptosis and necrosis in PMN and macrophages (Ragland *et al*. 1994). Upon infection with *Trypanosoma cruzi*, $CD4^+$ lymphocytes are killed by apoptosis and, therefore, $CD4^+$ T cell depletion could contribute to parasite persistence in Chagas' disease (Lopes *et al*. 1995). Similarly, in malaria caused by *Plasmodium falciparum* increased programmed cell death occurs in mononuclear cells (Toure-Blade *et al*. 1996). Taken together, in parasite infections apoptosis might play an important role in immunosuppression and the development of the diseases.

CONCLUDING REMARKS

Pathogens overcome the microbicidal activities of macrophages by either surviving intracellularly or by killing the immune cell. A strategy increasingly found to be employed by bacteria and parasites is to provoke apoptosis in macrophages. Thus, the phagocyte is efficiently killed since its pre-established cell death arsenal can be exploited. The best studied example of bacteria-induced apoptosis is *Shigella flexneri* which has evolved a peculiar host–pathogen relationship in that it does not establish a homeostatic condition within macrophages but rapidly kills this cell through apoptosis. However, although initially successful in overcoming the host's innate immune response, shigellosis is self-limiting in healthy individuals presumably due to an acute inflammation which is the direct aftermath of IL-1β secretion by the dying macrophage. Apoptosis as a trigger of inflammation might represent a novel mechanism in the establishment of complex host-pathogen relationships. Apart from surviving the macrophages, antibacterial activities, *Shigella's* benefit of evolving IpaB as a potent macrophage toxin could be that the bacteria can efficiently spread to other hosts because of diarrhoea.

On the other hand, among a family of ICE/CED-3 related cysteine proteases involved in programmed

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cell death, ICE might have particularly evolved as the trigger component of defensive apoptosis which upon stimulation specifically elicits a disease-limiting inflammation via release of mature IL-1β.

In recent years tremendous progress has been made in identifying molecular players of programmed cell death (Fraser & Evan, 1996). Mechanisms and regulation of apoptosis, however, still remain largely unsolved. Since IpaB induces apoptosis by directly binding to ICE or a related protease, this bacterial invasion might turn out to be a valuable tool in dissecting the delicate balance between the life and death of a cell.

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