

MINIREVIEW

Apoptosis and the Balance of Homeostatic and Pathologic Responses to Protozoan Infection

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Apoptosis, or programmed cell death (PCD), is a physiological response that eliminates unwanted cells, an evolutionarily ancient process that is present in all multicellular organisms. Apoptosis is used during embryogenesis as a way to shape future adult forms (e.g., limbs, fingers) or to suppress vestigial embryonic structures (e.g., genital organ involution during mammalian sexual differentiation). By eliminating unwanted cells, PCD is an important tool that provides space for new cells during the process of cell renewal (41). In higher vertebrates, apoptosis is employed to eliminate self-reactive lymphocytes during differentiation and again at the termination of an immune response to remove antigen-specific lymphocytes (3). The advantage of apoptosis over necrosis is the lack of an inflammatory response after cell death. Indeed, apoptotic cells do not release their contents into the milieu but shrink and are phagocytosed by tissue macrophages or dendritic cells (42).

Apoptosis is a continually occurring, tightly regulated process that maintains the homeostatic cellular balance in a normal animal. PCD is relatively rarely directly observed in healthy animals. This is because apoptotic cells are potent triggers for phagocytosis and thus are rapidly removed from the environment. Nevertheless, recent studies have revealed high levels of host cell apoptosis associated with several protozoan infections, particularly among cells of the immune system. In this review we address how protozoan infection triggers apoptosis and consider the consequences of dysregulated PCD for the parasite and the host.

MOLECULAR PATHWAYS UNDERLYING PCD

Apoptosis centers on proteolytic activation of a family of cysteine proteases known as caspases (Fig. 1). The caspase targets comprise a variety of proteins involved in cell survival, including DNA repair, cell cycle control, and nuclear and cytoskeletal assembly, and these enzymes also target downstream caspases in the cascade leading to apoptosis. The caspases are present as proenzymes in unstimulated cells. During activation, an N-terminal 3- to 24-kDa caspase prodomain is cleaved, and the remaining enzyme is further divided into a large subunit (17 to 21 kDa) and small subunit (10 to 13 kDa), which to-

gether form the active molecule. There are 14 known mammalian caspases. These molecules are divided into initiator and effector groups that act in cascade, with the former activating the latter by specific cleavage and the latter processing other cellular substrates. Caspase-8 (Casp-8) and Casp-9 activation cascades converge on Casp-3, which in turn activates the downstream effector caspases, Casp-6 and Casp-7 (49). Nevertheless, once triggered, the downstream effector caspases can activate initiator caspases, resulting in a potent positive feedback loop (60). Poly(ADP-ribose) polymerase, a 116-kDa nuclear protein involved in DNA repair, cell survival, and proliferation and differentiation mechanisms, is a major target substrate of caspases. Western blot detection of the signature p89 poly(ADP-ribose) polymerase fragment or other smaller fragments resulting from caspase cleavage is widely used to reveal apoptotic death in cells, tissues, and organs (14).

Initiator caspases are activated by two general mechanisms; the first involves ligation of cell surface receptors (in the case of Casp-8), and the second is a result of an internal, stress-mediated stimulus (in the case of Casp-9). Casp-8 is activated through a cell membrane receptor of the CD95 (Fas/Apo1) or CD120 (tumor necrosis factor [TNF] receptor) family which, after binding its relevant ligand (Fas or TNF- α), trimerizes and attracts the docking proteins Fas-associated death domain (FADD) and TNF receptor-associated death domain (TRADD) (Fig. 1A). The latter molecules recognize death domains contained within the receptor molecules themselves. FADD contains two death effector domains that directly bind and cleave Casp-8. TRADD lacks death effector domains but can activate Casp-8 by first binding FADD (65). Casp-8 has been shown to be essential for Fas-induced apoptosis (28) (Fig. 1A).

Casp-9 activation involves changes in the membrane potential of the mitochondria, resulting in cytochrome *c* translocation from the inner mitochondrial membrane to the cytoplasm (Fig. 1B). Loss of mitochondrial membrane potential can be induced by toxic agents, excess reactive oxygen and nitrogen intermediates, stress induced by UV or gamma irradiation, or growth factor deprivation. Cytoplasmic cytochrome *c* binds to apoptotic protease-activating factor 1 (APAF-1), resulting in a conformational change in the latter molecule. In presence of ATP, activated APAF-1 binds to procaspase 9, cleaving it to an active Casp-9 form (21). Furthermore, a change in the mitochondrial membrane potential and release of cytochrome *c* into the cytoplasm are tightly regulated by Bcl-2 family pro-

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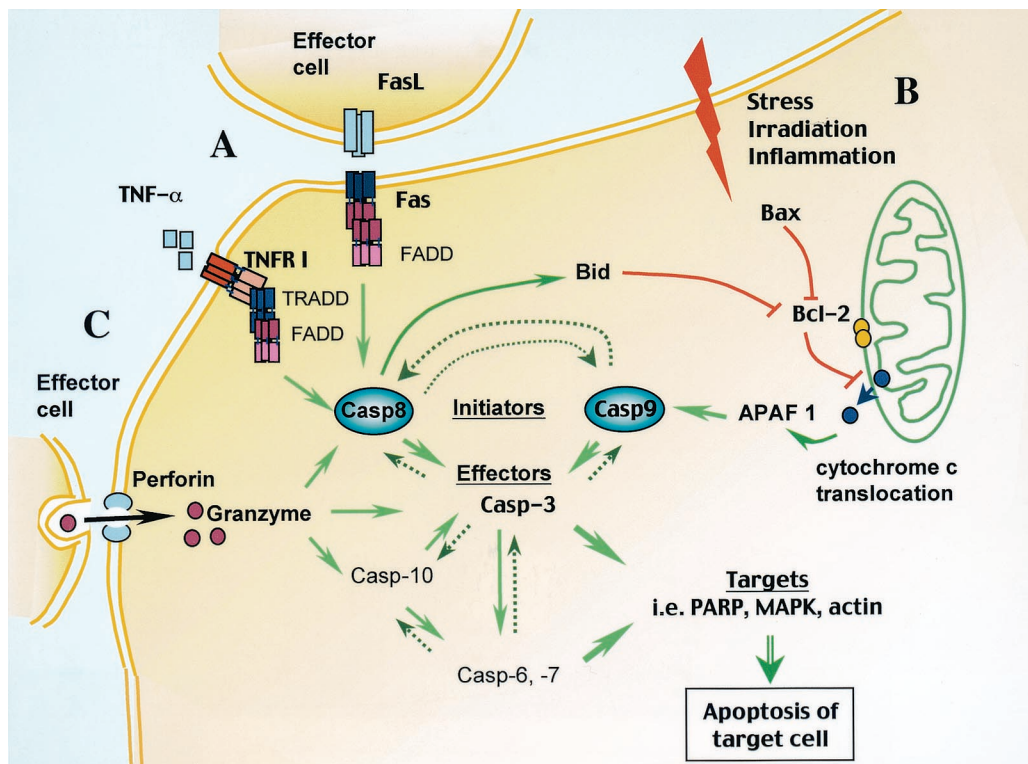


FIG. 1. PCD induced through multiple pathways. After cleavage and activation (green arrows) of the initiator Casp-8 and -9, the caspase cascade leads to cell death through further activation of death effector molecules. (A) Receptor-mediated induction of apoptosis. Extracellular molecules (TNF- α and FasL) bind to TNFR family members (TNFR I and Fas, respectively). Recruitment of FADD to the receptors leads to Casp-8 activation, which in turn results in activation of Casp-3, -6, and -7. (B) Stress, irradiation, and inflammation act on mitochondria through intermediary proapoptotic Bcl-2 family members such as Bax, resulting in blocking of the antiapoptotic activity of Bcl-2 (red lines). As a result of the latter event, cytochrome *c* translocates into the cytoplasm and activates Casp-9 through APAF-1. Casp-8 may also trigger the mitochondrial PCD pathway through activation of Bid, which, like Bax, inhibits the antiapoptotic activity of Bcl-2. (C) Cytotoxic cells introduce granzyme molecules into the target cell in a process mediated by multimerization of the perforin molecule. Granzymes cleave various substrates, including caspases, resulting in cell death. The dashed arrows indicate secondary effects of activated caspases. PARP, poly(ADP-ribose) polymerase; MAPK, mitogen-activated protein kinase.

teins that act by dimerization in the outer mitochondrial membrane. Some of these proteins are antiapoptotic (Bcl-2 and Bcl-XL), while others are proapoptotic (Bax and Bad), and the ultimate outcome eventually depends on the ratio of homodimers and heterodimers of each kind (2) (Fig. 1B).

Recently, a link between Casp-8 and Casp-9 activation was revealed by discovery of Bid, a small 23-kDa proapoptotic Bcl-2 family member. Upon activation of Casp-8 by an extracellular cell death signal, Bid is cleaved to a 15-kDa active fragment that translocates to the mitochondrial membrane. Through interaction with Bax, mitochondrial membrane depolarization and cytochrome *c* release occur, resulting in Casp-9 activation (38, 66, 69). Thus, Bid activation bypasses the need for mitochondrial stress factors in activation of Casp-9.

APOPTOSIS DURING THE IMMUNE RESPONSE

PCD is an important process during the normal immune response to infection. Cytolytic T lymphocytes employ perforin and granzyme molecules to directly trigger apoptosis in infected target cells (Fig. 1C). The mechanism involves perforin-dependent introduction of granzymes into the target cell cytoplasm. Granzyme B, the best-characterized granzyme, activates

caspases, such as Casp-10 and -7 (24, 62) (Fig. 1C). In addition, granzyme B may directly cleave a variety of intranuclear factors, resulting in caspase-independent apoptosis. More recently, granzyme B has been shown to directly cleave Bid to a 14-kDa fragment. This peptide, while distinct from the 15-kDa Bid fragment generated by Casp-8, displays similar functions in that it translocates to the mitochondrial membrane, attracts Bax, and thus mediates cytochrome *c* release and Casp-9 activation (22).

Several intracellular protozoans elicit major histocompatibility complex class I-restricted CD8⁺ cytotoxic T-lymphocyte (CTL) activity; these protozoans include *Toxoplasma gondii* (19, 61), *Plasmodium* spp. (4, 5, 56), *Trypanosoma cruzi* (51), *Leishmania major* (11), and *Encephalitozoon cuniculi* (33). Studies with perforin knockout (KO) mice support the hypothesis that CTL activity plays a limited role in host protection against *T. cruzi* and *T. gondii* infection (13, 50). In the case of *Toxoplasma*, human CD4⁺ CTL clones have also been isolated (10, 54).

PCD also plays an important role in homeostasis of the immune system through antigen-induced apoptosis of mature T and B cells at the termination of an immune response. This

process, termed activation-induced cell death (AICD), although well established as an essential component of the immune response, is not well understood and is likely to encompass several mechanisms. Strong antigenic stimulation has been reported to downregulate Bcl-2 and upregulate FasL, leaving cells sensitive to Fas-mediated death. Proximity to activated TNF- α -producing cells renders lymphocytes sensitive to TNF receptor I (TNFR-I)-induced apoptosis. AICD has also been demonstrated in vitro to be a response of mature T cells to T-cell receptor-CD3 triggering in the absence of costimulatory signals. Finally, activated T cells may upregulate CTLA-4 while simultaneously downregulating CD28. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) is then capable of delivering proapoptotic signals after CD80/CD86 triggering, instead of the survival signals that normally accompany CD28-CD80/CD86 ligation (27). The total effect of these processes is to remove clonally expanded effector cells that are no longer needed once an infection has been resolved. Apoptosis is the mechanism that does this without the complications of an inflammatory response that occur during cell necrosis.

Direct evidence that AICD occurs during protozoan infection comes from studies of acute murine toxoplasmosis. In this disease, CD4⁺ T lymphocytes become unresponsive at the peak of infection, and AICD can be triggered by in vitro restimulation of CD4⁺ T lymphocytes from the animals (32). Similarly, splenocytes from *T. cruzi*-infected mice can be induced to undergo AICD in response to in vitro mitogenic stimulation (36). The mechanisms underlying AICD during these and other protozoan infections may involve TNF- α -TNFR I and Fas-FasL interactions (see below).

TNF- α -TNFR I PATHWAY

Clearance of cellular infiltrates, presumably by phagocytosis of apoptotic cells, has been linked to the TNF- α -TNFR I pathway during experimental infections with *L. major* and *T. cruzi*. Infection of TNFR I (p55) gene KO mice leads to development of nonhealing lesions at the site of infection, although the animals retain the ability to clear parasites (64). Footpad lesions in KO mice have a major infiltrate of lymphocytes along with focal epidermal necrosis and surface ulcerations. Similar results were obtained with wild-type (WT) mice by antibody (Ab) neutralization of TNF- α (29). In addition, cells isolated from *L. major* lesions in WT mice are hypersensitive to TNF- α -mediated apoptosis in vitro. Thus, in the *L. major* infection model, the TNFR I pathway triggers cell death in lymphocyte infiltrates at the site of infection, and this plays a role in resolving the lesion. Nevertheless, TNF- α ^{-/-} animals display a phenotype distinct from that of mice lacking TNFR I (67). Thus, TNF KO animals infected with *L. major* rapidly succumb to progressive visceral leishmaniasis. The results suggest either that TNF binds to additional receptors or that the course of infection varies depending on the *L. major* strain.

Infection of TNFR I KO mice with *T. cruzi* results in increased CD45⁺ cellular infiltrates in myocardial tissue, suggesting that TNF- α also plays an important role in control of inflammation in the context of this parasite (7). Unlike *L. major* infection, the absence of signaling through TNFR I leads to uncontrolled parasitemia and decreased parasite killing in myocardial fibroblasts after in vitro interleukin-1 (IL-1) and

gamma interferon (IFN- γ) stimulation. However, WT and KO mice have comparable levels of apoptotic cells in the inflammatory lesions. This would not be predicted based upon the hypothesis that TNF- α -mediated apoptosis contributes to lesion resolution by triggering PCD. Possibly, the abnormally high parasitemia in TNFR I KO mice triggers other apoptotic pathways that do not occur during WT *T. cruzi* infection.

When *T. gondii* infection was studied, we and other workers found high levels of apoptotic death in the spleens of mice that experienced acute infection with high-virulence parasites, and analysis of infected TNFR I KO mice revealed partially reduced apoptosis (17, 18, 45). Because PCD was not totally eliminated in the absence of TNFR I, the results suggest that induction of PCD by high-virulence *T. gondii* involves activation of multiple pathways, including the TNF- α -TNFR I pathway.

FAS-FASL PATHWAY

The Fas-FasL pathway has no known function other than to mediate PCD. This contrasts with TNF- α -TNFR I signaling, which can have multiple effects in addition to PCD. Therefore, in vivo consequences of disrupting Fas-FasL signaling can be directly attributed to alteration of steady-state apoptosis.

Similar to findings obtained with TNFR I^{-/-} mice, *L. major* infection of *lpr* and *gld* mutant mice (which lack functional Fas and FasL, respectively) resulted in lesions that failed to resolve (9, 25). The lack of functional Fas or FasL resulted in increased numbers of parasites despite normal or even greater expression of IFN- γ and nitric oxide, factors usually linked to resistance. When the Fas-FasL pathway was reconstituted in *gld* mice by administration of recombinant soluble FasL, the animals resolved their lesions and eliminated the parasites. These studies provide clear evidence that FasL-triggered apoptosis plays a role in resistance to protozoan infection. However, whether this process involves lysis of infected macrophages or prevention of uncontrolled inflammatory reactions at the site of the lesion is not clear.

In a recent study of human patients infected with the visceral parasite *Leishmania donovani*, elevated levels of soluble Fas and FasL were found in sera, and spleen cell aspirates contained similarly increased amounts of membrane Fas and FasL (15). Additionally, in vitro infection of macrophages with *L. donovani* resulted in increased membrane-bound Fas expression and release of soluble FasL. These results suggest that Fas-FasL interactions may be an important component in human leishmaniasis.

CD4⁺ T lymphocytes from *T. cruzi*-infected mice express high levels of Fas and FasL mRNA and are sensitive to T-cell receptor-CD3-induced AICD in vitro. PCD in these cells is dependent upon the Fas-FasL pathway, since cells from *gld* mice are not sensitive to AICD (37). In cocultures of infected macrophages and T cells, parasite replication increases as T cells undergo Fas-mediated AICD (53). Indeed, interaction of apoptotic cells with *T. cruzi*-infected macrophages has been shown to fuel parasite growth, and this has been shown to be dependent upon synthesis of transforming growth factor β , prostaglandins, and polyamines (16).

Nevertheless, during in vivo *T. cruzi* infection, loss of the Fas-FasL pathway results in increased parasitemia and ele-

vated IL-4 and IL-10 production (37). Therefore, while the Fas-FasL pathway sensitizes CD4⁺ T cells to AICD and increased parasite replication in vitro, in an in vivo situation this pathway is required to maintain an intact Th1 response to *T. cruzi*, and disruption of the pathway leads to emergence of a Th2-dominant response and increased susceptibility.

The Fas-FasL pathway also has effects on B lymphocytes during *T. cruzi* infection. Up to 18% of splenic B cells from infected animals have been reported to undergo apoptotic death during acute murine *T. cruzi* infection (1). These cells display increased levels of both Fas and FasL, expression of which is positively correlated with the activation status of the cells. The Fas-FasL-bearing B cells also express membrane antiparasite immunoglobulin G. Apoptosis of the activated B cells could be prevented in vitro by addition of anti-FasL Ab and was associated with AICD through Fas-FasL B cell-B cell interactions in a process termed fratricide. Thus, apoptosis through the Fas-FasL pathway specifically targets *T. cruzi*-restricted immunoglobulin G-producing B cells in this model, resulting in weakened antiparasite humoral immunity.

T-cell apoptosis can also be restricted to the cells specific for parasite antigen. When ovalbumin- or parasite-specific T cells are adoptively transferred into *Plasmodium yoelii*-infected T-cell-deficient nude BALB/c mice only the *P. yoelii*-specific T cells undergo PCD (68). Parasite-specific T cells display increased Fas expression, which implicates the Fas-FasL pathway of apoptosis in this model. The results suggest that there is a parasite strategy of eliminating anti-*Plasmodium* T-cell effectors. Moreover, up to 60% of the spleen cells express Fas during the acute phase of infection with blood stage *Plasmodium chabaudi chabaudi* AS, showing in this case a much more generalized sensitivity to the parasite (23).

In humans infected with *Plasmodium falciparum*, high levels of apoptotic markers are found in peripheral blood mononuclear cells. The cells are sensitive to AICD induced in vitro, and the levels of serum-soluble FasL are elevated. The levels of FasL decrease after anti-*Plasmodium* chemotherapy (30). Thus, Fas-FasL-induced apoptosis may also be an important component of human malaria.

In the absence of NF- κ B2, mice succumb to *T. gondii* infection and display increased splenic apoptosis and elevated expression of Fas. Thus, *T. gondii* may trigger Fas-FasL-dependent apoptosis, in a process under the negative control of NF- κ B2 (6). During intraocular infection with a low-virulence *T. gondii* strain, expression of Fas and FasL is increased in the inflamed retina, as well as in the spleen. The presence of apoptotic cells in the eyes of infected WT mice is associated with pathology and is absent in *gld* and *lpr* infected mice. Nevertheless, Fas- and FasL-defective animals display increased inflammatory infiltrates and higher clinical scores. These results are reminiscent of the results obtained for *L. major* infection of TNFR KO mice, in which apoptosis is employed as a mechanism to remove unwanted inflammatory cells. After peroral *Toxoplasma* infection, susceptible mouse strains develop a lethal inflammatory gut pathology accompanied by Fas-dependent apoptosis of Peyer's patch T cells, which is probably the result of inflammatory cytokine overproduction (35). We found generalized apoptosis in the spleens of infected mice, a phenomenon partially dependent upon Fas-

FasL interaction, as shown by the lack of apoptotic cells in *gld* infected animals (18).

Similar to *Toxoplasma* infection in the gut, infection with *Cryptosporidium parvum* elicits death of intestinal epithelia through a Fas-FasL-dependent pathway. Indeed, in vitro infected epithelial cells upregulate cell surface FasL, while increased FasL mRNA levels are detected in intestinal epithelia during in vivo infection (46). *C. parvum*-infected, CD4-depleted *gld* and *lpr* mice display reduced weight loss compared to CD4-depleted WT animals, which clearly implicates Fas-FasL-dependent apoptosis in the pathogenesis of *C. parvum* infection.

MITOCHONDRION-INDUCED APOPTOSIS

Mitochondrion-dependent apoptosis during infections with both *T. gondii* and *Plasmodium berghei* has been documented recently. During acute *T. gondii* infection, CD4⁺ and CD8⁺ T lymphocytes accumulating in the brain expressed increased FasL, Bax, and Bcl-2 mRNA levels (58). The latter two gene products are associated with mitochondrion-dependent apoptosis (Fig. 1B). During chronic infection, T-lymphocyte expression of proapoptotic molecules is downregulated, which is accompanied by increased levels of antiapoptotic Bcl-2 and Bcl-XL. When restimulated in vitro, T cells do not proliferate, but they upregulate expression of the cell cycle inhibitors p21Waf/cip1 and p27kip1. The data suggest that low-level apoptosis plays a role in gradually decreasing the size of the pool of intracerebral T cells during chronic infection. We also found increased expression of both Bax and Bcl-2 in the spleens of mice experiencing acute infection with high-virulence parasites, although it was difficult to correlate this with either a pro- or antiapoptotic outcome (unpublished observations).

In a recent study, mice experiencing lethal infection with *P. berghei* were similarly found to express increased levels of Bax, Bcl-2, p53, and cytoplasmic cytochrome *c* in the brain (34). Furthermore, mitochondria analyzed by electron microscopy displayed an abnormal morphology suggestive of late-stage degeneration. While it is not clear whether in this case mitochondrion-dependent PCD was triggered through intrinsic or extrinsic apoptotic cascades, dysfunction of this cellular organelle and the subsequent death of the cell may be important components of cerebral malaria.

INFLAMMATORY TRIGGERS OF APOPTOSIS

Several studies have linked production of proinflammatory mediators with PCD during *T. gondii* infection (17, 35, 45). Furthermore, apoptosis resulting from inflammatory cytokine overproduction is associated with high-virulence *Toxoplasma* strains but not with low-virulence *Toxoplasma* strains (17, 45). The widespread apoptosis associated with acute *T. gondii* infection is greatly reduced in mice lacking expression of either IFN- γ or IL-12 (18) or treated with anti-IFN- γ Abs (35). Similarly, treatment of *T. cruzi*-infected mice with either anti-IFN- γ Ab or anti-TNF- α Ab reduced apoptosis (39), while cells infected with *Neospora caninum* were sensitive to IFN- γ -mediated apoptosis (52). The proapoptotic effects observed were most probably the result of the ability of IFN- γ to upregulate expression of the Fas-FasL and TNF- α -TNFR I path-

ways (35). Interestingly, induction of apoptosis was correlated with STAT-1 activation during *T. gondii* infection (18).

Nitric oxide has also been implicated as an apoptotic trigger during *T. gondii* and *T. cruzi* infections. An absence of NO in gene-targeted or drug-treated mice reduces the levels of splenocyte apoptosis that normally occur during these infections (18, 39, 40, 55). The mechanism by which NO induces PCD is not clear, but it may involve the effects of peroxynitrite formation from NO and superoxide in mitochondria (43).

It is difficult to determine the biological significance of apoptosis driven by proinflammatory mediators such as IFN- γ , IL-12, NO, and TNF- α . Because these molecules are involved in resistance to microbial pathogens, their presence is often required for host survival. Thus, Ab depletion and gene KO experiments may well reveal a role for proinflammatory mediators in pathogen-triggered PCD. Nevertheless, in these situations host death occurs as a result of uncontrolled parasite replication and tissue damage.

PROTOZOAN MOLECULES THAT DIRECTLY TRIGGER APOPTOSIS

In many cases the cell death that accompanies protozoan infection results from the effects of host inflammatory mediators. However, several studies have pointed to factors produced by protozoan parasites that directly activate apoptotic pathways in target cells. Thus, *Entamoeba histolytica* was found to induce contact-dependent apoptosis in epithelial cell cultures. In this case apoptosis is Fas-FasL and TNFR I independent. The response is Casp-3 dependent, but interestingly it does not appear to require either Casp-8 or Casp-9 (26, 59). Host protein dephosphorylation, associated with phosphatase PTP1B activation, was recently reported in *E. histolytica*-infected Jurkat cell cultures and was found to be parasite contact dependent. Infected Jurkat cells undergo apoptosis, but they are rescued in the presence of phosphatase inhibitors (63). These results indicate that host protein dephosphorylation may be involved in Casp-3 activation leading to PCD.

T. cruzi induces strong but transient cell depletion in the thymus during acute Chagas' disease. The effect appears to be the result of parasite *trans*-sialidase (TS) shedding in the blood, resulting in direct apoptosis of the thymic nurse cell complex mediated by this virulence factor, as treatment with anti-TS Ab eliminates the effect (47). TS-induced T-cell depletion may therefore represent a *T. cruzi* strategy to avoid host immunity. Paradoxically, *T. cruzi* TS has an opposite, antiapoptotic effect on human Schwann cells, the principal target in the peripheral nervous system, apparently through a pathway dependent upon a phosphatidylinositol 3-kinase–Akt signaling cascade (8).

CONCLUSION: IS APOPTOSIS GOOD OR BAD?

It is clear that activation of PCD pathways contributes to the pathology of several protozoan infections (Table 1). Less clear is whether apoptosis is a beneficial or detrimental response in these situations. The correct answer is probably dependent upon the particular protozoan and even the particular model system. In many cases, it is difficult to determine whether the host or parasite (or neither) benefits from host cell death. This

TABLE 1. Outcomes of apoptotic cell death during protozoan infections

Protozoan	Mechanism	Reference(s)
Homeostatic outcomes		
<i>T. gondii</i>	CTL	10, 12, 19, 31, 54
	Bcl-2 family	58
	NO	55
<i>Plasmodium</i> spp.	CTL	4, 5, 56
	<i>L. major</i>	11
	TNFRI	29, 64
<i>T. cruzi</i>	Fas-FasL	9, 25
	CTL	51
	TNFRI	7
	Fas-FasL	37
Pathogenic outcomes		
<i>T. gondii</i>	AICD	32, 36
	Proinflammatory cytokines	17, 45
	Fas-FasL	35
<i>L. donovani</i>	Fas-FasL	15
<i>T. cruzi</i>	AICD	53
	NO	39
	Fas-FasL	40
	Parasite factor	47
<i>Plasmodium</i> spp.	AICD	68
	Fas-FasL	23, 30
	Bcl-2 family	34
<i>C. parvum</i>	Fas-FasL	46
<i>N. caninum</i>	IFN- γ	52
<i>E. histolytica</i>	Parasite factor	26, 59, 63

is because the same cytokines that trigger apoptosis also are necessary to promote antimicrobial effector functions. Removal of the cytokines, either by genetic inactivation or by Ab depletion, may eliminate parasite-induced PCD. Nevertheless, the result may be uncontrolled microbial proliferation, causing pathology in affected tissues and frequently host death.

Studies with *Leishmania* provide a clear example of a situation in which PCD plays an important role in lesion resolution following control of infection. *T. cruzi* TS appears to provide an example of a proapoptotic parasite molecule whose purpose is to suppress host immunity and promote infection. The purpose of host cell death during *T. gondii* and *Plasmodium* spp. infections is more obscure. Here, apoptosis may be the accidental consequence of the inflammatory cytokine response to infection, a response otherwise needed to allow host survival. A more interesting possibility is the possibility that apoptosis is actively induced by *T. gondii* and other protozoans and may therefore promote infection by removing effectors of the immune system.

Not considered in this review is the other side of the coin, the possibility that some microbial pathogens suppress apoptosis. For example, macrophages infected with *Toxoplasma* are resistant to multiple inducers of apoptosis, and this is associated with decreased caspase activation and reduced poly(ADP-ribose) polymerase expression (20, 48). *L. donovani*-infected macrophages display increased viability in the absence of growth factors, and this is the result of soluble mediators released by infected cells (44). For protozoan pathogens such as these organisms, apoptosis during in vivo infection is the net result of both pro- and antiapoptotic effects on the host.

Induction of apoptosis during protozoan infection can be

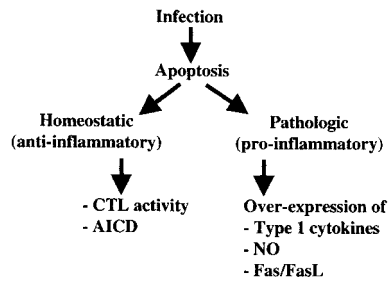


FIG. 2. Apoptosis during protozoan infection. From the host perspective, CTL activity and AICD can be viewed as homeostatic, anti-inflammatory responses to protozoan parasite infection that control the spread of infection and resolve the immune response after resolution of the infection. Alternatively, overexpression of type 1 proinflammatory cytokines, nitric oxide, or Fas-FasL may lead to uncontrolled apoptosis, in this situation becoming a proinflammatory response. A switch from a homeostatic response to a pathological response may occur in the course of infection, as when type 1 cytokines act to limit infection but then cause tissue and cellular apoptotic damage.

viewed from two angles (Fig. 2). First, it is a homeostatic mechanism that acts as a stabilizing factor in the host-parasite relationship. Both CTL activity and AICD contribute to this process, which, in general, decreases inflammatory responses by immunologically silent removal of infected and unwanted cells. The effect is to promote host survival, which also benefits the parasite, although CTL activity could potentially result in complete clearance of the pathogen. Second, apoptosis may be a destabilizing factor in the encounter between host and parasite. In this case, dysregulated PCD can be a component of a pathology resulting from inflammatory cytokine overproduction (Fig. 2). In this case, and like *Shigella* and *Salmonella* infections (57, 70), apoptosis becomes a proinflammatory phenomenon. The outcome of such inflammatory pathology could be detrimental to both the host and the parasite. It may also promote parasite spread by interfering with host defense, or alternatively, it may be a necessary cost incurred by the host during the process of controlling infection. Insights into the molecular pathogenesis of proapoptotic protozoans can be expected to lead to novel therapeutic intervention strategies to control microbial infections.

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