Pro- and anti-apoptotic activities of protozoan parasites

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SUMMARY

During infection, programmed cell death, i.e. apoptosis, is an important effector mechanism of innate and adaptive host responses to parasites. In addition, it fulfils essential functions in regulating host immunity and tissue homeostasis. Not surprisingly, however, adaptation of parasitic protozoa to their hosts also involves modulation or even exploitation of cell death in order to facilitate parasite survival in a hostile environment. During recent years, considerable progress has been made in our understanding of apoptosis during parasitic infections and there is now convincing evidence that apoptosis and its modulation by protozoan parasites has a major impact on the parasite-host interaction and on the pathogenesis of disease. This review updates our current knowledge on the diverse functions apoptosis may fulfil during infections with diverse protozoan parasites including apicomplexans, kinetoplastids and amoebae. Furthermore, we also summarize common mechanistic themes of the pro- and anti-apoptotic activities of protozoan parasites. The diverse and complex effects which parasitic protozoa exert on apoptotic cell death within the host highlight fascinating interactions of parasites and their hosts. Importantly, they also stress the importance of further investigations before the modulation of host cell apoptosis can be exploited to combat parasitic infections.

Key words: Apoptosis, signal transduction, parasite-host interaction, immunity, immune evasion, protozoa, pathogenesis.

INTRODUCTION

More than a decade ago, the pioneering work of Moore and Matlashewski (1994) first indicated that protozoan parasites, e.g. *Leishmania donovani*, manipulate apoptotic cell death of their host cells as previously described for viruses and bacteria (for a comparison see Benedict, Norris and Ware, 2002; Häcker and Fischer, 2002). Since then, the number of parasites which are known to modulate apoptosis within their hosts has continuously expanded and currently includes a variety of protozoans of major medical impact on human health and livestock production (Table 1). Recently, manipulation of the host apoptotic cell death has also been expanded to infections with helminths, e.g. filariae and schistosomes (Chen *et al*. 2002; Jenson *et al*. 2002). However, these interactions are beyond the scope of this review. Different protozoa exert diverse effects on the host cell's fate including induction and inhibition of apoptotic cell death as well as simultaneously triggering both pro- and anti-apoptotic activities (reviewed in Heussler, Küenzi and Rottenberg, 2001*a*; Lüder, Gross and Lopez, 2001). In the case of intracellular parasites, this parasite–host interaction becomes even more heterogeneous, since manipulation of apoptosis can either affect the infected host cell or uninfected bystander cells. Furthermore, with the unexpected observation of apoptosis or apoptosis-like cell death not only in metazoan parasites but also in protozoans (Ameisen, 1996; Welburn, Barcinski and Williams, 1997; Deponte and Becker, 2004), parasitologists, cell biologists and immunologists have to consider an additional level of complexity in the interplay between parasites and apoptosis.

Given the wide distribution of manipulating apoptotic cell death within their hosts, it is tempting to speculate that such parasite–host interactions play a crucial role for the parasitic life style. Indeed, during recent years a variety of functions have been attributed to the modulation of host cell apoptosis by protozoan parasites, although some of these may just represent bystander effects. In either case, apoptosis and its modulation during infection have been shown to play important roles in the outcome of infection or the pathogenesis of disease. It is this impact on the course of infection that makes apoptotic cell death during parasitic infections a rapidly expanding area of ongoing research. Taking the considerable progress being made in unravelling the molecular pathways regulating apoptosis (Hengartner, 2000) as well as the parasite mechanisms to 'hijack' apoptosis-regulating cascades of its host cells, apoptosis might become a promising target to combat parasitic diseases. Additionally, knowledge of the functions and the mechanisms of apoptosis during parasitic infections may also provide tools in the development of effective anti-parasitic vaccines as recently hypothesized (James and Green, 2004).

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Parasitology (2006), **132**, S69–S85.
doi:10.1017/S0031182006000874 Printed in the United Kingdom

Table 1. Manipulation of host cell apoptosis by protozoan parasites

Parasite species	Modulation	Affected cell type	Putative function(s)	References
			Intracellular parasites	
Leishmania spp.	Delayed	Neutrophils	Vector for macrophage entry	Aga *et al*. 2002; Van Zandbergen *et al*. 2004
	Inhibited	Macrophages	Intracellular survival	Moore *et al*. 1994
	Enhanced	T lymphocytes	Evasion of systemic immunity	Das *et al*. 1999; Bertho *et al*. 2000
P. falciparum	Induced	Endothelial cells	?	Pino *et al*. 2003*a*; Hemmer *et al*. 2005
P. berghei	Inhibited	Hepatocytes	Intracellular survival	Leiriao *et al*. 2005; Van de Sand *et al*. 2005
Plasmodium spp.	Enhanced	T and B lymphocytes	Evasion of systemic immunity	Toure-Balde *et al*. 1995; Helmby *et al*. 2000
T. gondii	Inhibited	Various	Intracellular survival	Nash *et al*. 1998; Goebel *et al*. 1999, 2001
	Induced	Trophoblast cells	Dissemination	Abbasi *et al*. 2003
	Enhanced	T lymphocytes	Evasion of systemic immunity	Khan *et al*. 1996; Liesenfeld *et al*. 1997
T. cruzi	Enhanced	T and B lymphocytes	Supply of putrescine	Nunes *et al*. 1998; Freire-de-Lima *et al*. 2000
			Evasion of systemic immunity	Lopes *et al*. 1995; Zuniga *et al*. 2002
	Induced	Macrophages	Dissemination (?)	Freire-de-Lima *et al*. 1998
	Inhibited	Neurons, glia, myocytes	Intracelluar survival	Chuenkova *et al*. 2001; Hashimoto *et al*. 2005
Theileria spp.	Inhibited	T and B cells, macrophages	Colonization of host cells, intracellular survival	Heussler *et al*. 1999; Küenzi *et al*. 2003; Dessauge *et al*. 2005*b*
C. parvum	Inhibited	Intestinal epithelial cells	Intracellular survival	McCole *et al*. 2000; Chen *et al*. 2001
	Enhanced	Intestinal epithelial cells	?	Chen *et al*. 1999; McCole *et al*. 2000
			Extracellular parasites	
E. histolytica	Induced	Leukocytes, erythrocytes	Phagocytosis of host cells	Huston *et al*. 2003; Boettner *et al*. 2005
T. brucei	Induced	Endothelial cells	Crossing blood-brain barrier (?)	Girard *et al*. 2003; Stiles *et al*. 2004

IMPACT OF THE MODULATION OF APOPTOSIS DURING INFECTION

The different life-styles of protozoan parasites are crucial determinants for the effects exerted on host cell apoptotic cascades. Obligate intracellular parasites, for instance, rely on intact host cells to grow, propagate and differentiate. Thus, a variety of intracellular protozoans, including *Leishmania* spp., *Trypanosoma cruzi*, *Toxoplasma gondii* and *Theileria* spp. evolved mechanisms to suppress or delay apoptosis in infected host cells (Table 1). Such anti-apoptotic activities are indeed thought to be important prerequisites for sustained intracellular survival and development of parasites. Strikingly, however, despite its intracellular life style, *Cryptosporidium parvum*, a parasite of the intestinal epithelium, transiently induces apoptosis within the infected cell (Chen *et al*. 1999; Mele *et al*. 2004). Such host cell apoptosis correlates with the developmental stage of *C. parvum*, being only induced by sporozoites and meronts/merozoites, but not by trophozoites (Mele *et al*. 2004). The significance of increased apoptosis in *Cryptosporidium*-infected cells is still unknown; possible explanations include a partially successful 'natural' suicide programme of the host cell to limit parasite propagation or transient attenuation of the host's inflammatory response due to apoptosis instead of necrosis (McCole *et al*. 2000). Alternatively, induction of apoptosis during late stages of infection possibly facilitates dissemination and shedding of merozoites into the environment. In contrast to intracellular parasites, those residing in the extracellular environment do not rely on the integrity of certain host cells unless viability of the host is threatened. Extracellular parasites, such as, for example, *Trypanosoma brucei* or *Entamoeba histolytica* consequently rather induce than inhibit apoptosis (Table 1). In addition, apoptosis of immune cells may be triggered after infection with multiple parasites irrespective of their localization, thereby, evading efficient adaptive immune responses of the host (Table 1).

https://doi.org/10.1017/S0031182006000874 Published online by Cambridge University Press

Role of apoptosis during intracellular colonization of host cells

A crucial step in the life cycle of intracellular parasites is to gain entry into those host cells which are suitable for growth, differentiation and replication. It is now apparent that host cell apoptosis and modulation thereof plays a crucial role during host cell colonization by certain protozoa. Such strategies may be especially important for parasites with a restricted host cell spectrum as well as those relying on the phagocytic machinery of the host. Recently, *L. major* has indeed been shown to exploit polymorphnuclear neutrophil granulocytes (PMN) as 'Trojan horses' early after infection of the host to gain entry into their final host cells, i.e. the macrophages (Van Zandbergen *et al*. 2004). Since PMN are short lived and rapidly undergo apoptosis, *L. major* has evolved mechanisms to delay apoptotic cell death in parasite-infected cells in order to sustain PMN viability (Table 1; Aga *et al*. 2002). Importantly, *L. major*-infected PMN undergo apoptosis during later stages of infection and these cells are then readily phagocytosed by macrophages leading to a productive infection within the final host cell type of the parasite (Van Zandbergen *et al*. 2004). Exposure of 'eat-me' signals including phosphatidylserine (PS) on the outer leaflet of apoptotic PMN (Savill and Fadock, 2000), thereby, provides the appropriate signal for macrophages to engulf the dying host cell together with the intracellularly residing parasites.

T. parva and *T. annulata* employ another strategy to colonize their final host cells, i.e. T and B cells or macrophages (see also Heussler, Sturm and Langsley; this supplement). Schizonts of *Theileria* spp. induce an uncontrolled proliferation of parasitized leukocytes (reviewed by Dobbelaere and Heussler, 1999; Dobbelaere and Küenzi, 2004). During cell division, parasites disseminate to both daughter cells by attachment to the mitotic spindle. Thus, these unique abilities result in clonal expansion of *Theileria*-infected host cells. The parasite-induced 'tumorigenesis' may, however, require mechansims to avoid apoptosis, and elimination of *T. parva* by chemotherapy indeed leads to apoptosis of the cured T cells (Heussler *et al*. 1999). The ability to colonize their host cell population as well as to disseminate within the host therefore relies – among other mechanisms – on the ability to abolish host cell apoptosis (Table 1).

Impact of apoptosis on parasite dissemination and on the alteration of biological barriers

Erythrocytes containing trophozoites and schizonts of the human malaria parasite *Plasmodium falciparum* adhere to endothelial cells leading to sequestration, endothelial damage and eventually to severe complications including cerebral malaria. Recently, apoptotic endothelial cells have been detected *in vitro* as well as *in vivo* in fatal cases of human malaria indicating that apoptosis may be involved in damage to microvessels during life-threatening disease (Table 1; Pino *et al*. 2003*a*; Hemmer *et al*. 2005). Adherence involves – among other ligand-receptor interactions – also the binding of PS-exposing parasitized erythrocytes to CD36 and thrombospondin on endothelial cells (Eda and Sherman, 2002) which then leads to activation of both death receptor and mitochondrial apoptotic pathways (Pino *et al*. 2003*a*). Importantly, apoptosis of endothelial cells was shown to be particularly prominent in the presence of sera from patients with fatal or severe malaria (Hemmer *et al*. 2005). These observations further our understanding on the pathophysiology of severe malaria and may provide new therapeutic approaches against severe *P. falciparum* malaria (Hemmer *et al*. 2005).

Other protozoans might even exploit the apoptotic programme of the host in order to cross biological barriers. *T. brucei* ssp., the causative agent of human African trypanosomiasis, invades the central nervous system leading to the meningo-encephalitic stage of disease. For an extracellular parasite, crossing the blood-brain barrier is not trivial and may be facilitated by the induction of apoptosis in endothelial cells and perivascular glial cells. Recently, a soluble, parasite-derived 'trypanosome apoptotic factor' has been identified in sera from infected mice that indeed induce apoptosis in endothelial cells (Table 1; Stiles *et al*. 2004). Furthermore, apoptosis is also induced in endothelia and microglia by cerebrospinal fluids from African trypanosomiasis patients but not from control individuals suggesting that apoptosis might also affect the blood-brain barrier integrity in infected patients (Girard *et al*. 2003). Whether and to what extent this enables *T. brucei* to cross the blood-brain barrier, however, remains to be elucidated.

T. gondii was shown to induce apoptosis in primary human trophoblast cells *in vitro* (Abbasi *et al*. 2003). Interestingly, apoptosis was only observed in parasite-negative, but not in parasite-positive cells, indicating that the parasitic pro-apoptotic activity is blocked by the presence of intracellular parasites (see below). These results, nevertheless, suggest that the induction of apoptosis in uninfected bystander cells is possibly involved in parasite dissemination to placental tissue and the infection of the fetus, thereby, leading to congenital toxoplasmosis (Table 1).

Apoptosis-assisted supply of nutrients and growth factors

Killing and subsequent phagocytosis of host cells including erythrocytes is a major virulence factor of *E. histolytica* and may provide the parasite with essential nutrients during colitis and invasive

https://doi.org/10.1017/S0031182006000874 Published online by Cambridge University Press

amoebiasis. It has been shown recently that parasite-induced apoptosis in target cells may be involved in this process (Table 1; Huston *et al*. 2003; Boettner *et al*. 2005) although necrotic cell death may also participate (Berninghausen and Leippe, 1997). Exposure of PS on the outer leaflet of apoptotic leukocytes or erythrocytes co-cultured with *E. histolytica* appears to increase phagocytic efficiency (Huston *et al*. 2003; Boettner *et al*. 2005). This indicates the involvement of a PS receptor on the surface of *E. histolytica* and establishes molecular characteristics of apoptosis as crucial for host cell engulfment.

The causing agent of Chagas' disease, *T. cruzi*, leads to activation-induced apoptosis of T lymphocytes upon infection, thereby, dampening the adaptive immunity against the parasite (Lopes *et al*. 1995). Interestingly, co-culture of *T. cruzi*-infected macrophages with apoptotic T cells exacerbates intracellular replication of the parasite (Nunes *et al*. 1998). Analyses of the underlying mechanisms revealed that apoptotic T cells trigger the release of prostaglandin E_2 (PGE_2) and transforming growth factor (TGF)-β by macrophages (Freire-de-Lima *et al*. 2000). This diminishes inflammatory responses including nitric oxide (NO) production and alters the macrophage physiology with increased ornithine decarboxylase (ODC) activity and putrescine production (Freire-de-Lima *et al*. 2000). Since ODC activity is a limiting step in polyamine synthesis and has not been detected in *T. cruzi* (Hernandez and Schwarz de Tarlovsky, 1999), increased putrescine levels after uptake of apoptotic T cells by infected macrophages may assist parasite growth and replication (Table 1). Importantly, therapeutic inhibitors of this pathway markedly inhibited parasite growth *in vitro* and *in vivo* and appear to help in controlling Chagas' disease (Freire-de-Lima *et al*. 2000).

Avoidance of the cellular suicide programme

Infection of cells by intracellular parasites may provide an appropriate stress signal to commit suicide via the mitochondrial apoptotic pathway (see below) thereby limiting development and spread of the parasite progeny. Apoptosis is thus, generally, viewed as an innate defence mechanism against intracellular pathogens (Williams, 1994; Everett and McFadden, 1999) and this has indeed been confirmed for viruses (Benedict *et al*. 2002). Only recently, has such a scenario been extended by the strength of experimental data to protozoan parasites. Indeed, genetically attenuated *P. berghei* sporozoites evoke apoptosis upon infection of hepatocytes, thereby, optimizing presentation of parasite antigens to the immune system (Van Dijk *et al*. 2005). It might, thus, be speculated that these parasites, although being able to invade hepatocytes, have lost their ability to inhibit host cell apoptosis. As a consequence, infected hepatocytes encountering these parasites react with their natural anti-microbicidal apoptotic programme. Whether intracellular parasites would, however, in general trigger the mitochondrial apoptotic pathway of their host cells, awaits future experimental proof.

The fact that a variety of intracellular protozoans has been shown to inhibit spontaneous host cell apoptosis or apoptosis induced by stress-related pro-apoptotic stimuli argues for a considerable selective advantage for those which are able to block the natural suicide programme (Table 1). Such parasites include *Leishmania* spp. (Moore and Matlashewski, 1994; Aga *et al*. 2002), *T. cruzi* (Chuenkova *et al*. 2001; Aoki *et al*. 2004), *T. gondii* (Nash *et al*. 1998; Goebel, Lüder and Gross, 1999; Goebel, Gross and Lüder, 2001; Payne, Molestina and Sinai, 2003), *C. parvum* (McCole *et al*. 2000; Chen *et al*. 2001), *Theileria* spp. (Heussler *et al*. 1999; Küenzi, Schneider and Dobbelaere, 2003; Dessauge *et al*. 2005*b*) and *P. berghei* (Leiriao *et al*. 2005; Van de Sand *et al*. 2005). The magnitude of inhibition may vary between different parasite species as well as between different host cell types. *T. gondii*, for example, completely blocks apoptotic cascades triggered by a wide range of pro-apoptotic stimuli including growth factor withdrawal, irradiation, transcriptional inhibitors, protein kinase inhibitors and others (Nash *et al*. 1998; Goebel *et al*. 2001). In contrast, infection of epithelial cells with *C. parvum* decreases chemically-induced apoptosis to intermediate levels and even induces moderate levels in untreated cells (McCole *et al*. 2000). This indicates that *C. parvum* is only partially able to counterbalance host cell apoptosis. Whether this benefits the host or the parasite is still unknown. On the other hand, *T. cruzi* has been shown to inhibit apoptosis in neurons, Schwann cells and cardiomyocytes (Chuenkova and Pereira, 2000; Chuenkova *et al*. 2001; Aoki *et al*. 2004) while inducing apoptosis in infected macrophages (Table 1; Freire-de-Lima *et al*. 1998). Since IFN-γ is required for *T. cruzi*-triggered apoptosis in macrophages, the host cell type and the microenvironment are crucial factors for the parasite ability to alter apoptosis.

Recently, significant progress has been made concerning the avoidance of the cellular suicide programme after parasitic infection *in vivo*. After intraperitoneal infections of mice with *T. gondii*, parasites were readily deteced in non-apoptotic macrophages but were rarely found in those which undergo apoptosis (Orlofsky *et al*. 2002). The number of parasites was also substantially higher in non-apoptotic than in apoptotic cells indicating that avoidance of macrophage suicide after infection favours parasite replication (Orlofsky *et al*. 2002). Similarly, several days after infection with *L. major*, intact parasites were found intracellularly in viable, normally short-lived PMN suggesting that spontaneous apoptosis was inhibited (Aga *et al*. 2002).

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Although definite proof is still missing, these results clearly argue for an interference of at least certain parasites with host cell apoptosis *in vivo* as a means to facilitate intracellular survival.

Evasion of systemic immune reponses

Cytotoxic T lymphocytes and NK cells kill target cells by the induction of apoptosis via the release of toxic granule constituents or by ligation of death receptors, i.e. Fas/CD95 (see below). Similarly to the interference with the cellular suicide programme, intracellular protozoans may thus also rely on the ability to inhibit death receptor-induced cell death in order to develop or persist intracellularly. *T. gondii* (Nash *et al*. 1998), *T. parva* (Küenzi *et al*. 2003) and *T. cruzi* (Hashimoto, Nakajima-Shimada and Aoki, 2005) are indeed able to block apoptosis induced by cytotoxic lymphocytes (Table 1). Importantly, this explains why lymphocyte cytotoxicity obviously does not represent the primary effector mechanism to control parasite development during toxoplasmosis (Denkers *et al*. 1997) and Chagas' disease (Nickell and Sharma, 2000).

Besides representing an effector mechanism induced in target cells by cytotoxic leukocytes, apoptosis is crucial in the develoment and homeostasis of the immune system (Opferman and Korsmeyer, 2003). It is this function that makes apoptosis the most common form of cell death in T and B cells and a major regulator of immunity in response to infectious agents. Consequently, several parasites including *T. cruzi* (Lopes *et al*. 1995), *Leishmania* spp. (Das *et al*. 1999; Bertho *et al*. 2000), *T. gondii* (Khan, Matsuura and Kasper, 1996; Liesenfeld, Kosek and Suzuki, 1997) and *Plasmodium* spp. (Toure-Balde, Sarthou and Roussilhon, 1995; Helmby, Jönsson and Troye-Blomberg, 2000) have managed to employ such regulatory pathways in order to counterbalance systemic antiparasitic immunity (Table 1). Triggering lymphocyte apoptosis not only assists parasites to survive and eventually persist in immunocompetent hosts but also protects the host from overwhelming immunopathology. Defective cell death was indeed accompanied by increased inflammatory responses within ocular tissues after inoculation of *T. gondii* (Hu *et al*. 1999). Similarly, deficient T cell apoptosis correlated with massive lymphadenopathy and susceptibility to *L. major* infection whereas early T cell death and absence of lymphocyte accumulation occurred in resistant mice (Desbarats *et al*. 2000). Apoptosis of lymphocytes can be induced via a Fas-dependent 'activation-induced cell death' (AICD) or a Fas-independent passive cell death due to a 'neglect mechanism' (Krammer, 2000; Opferman and Korsmeyer, 2003). The fact that during experimental Chagas' disease, CD4+ T cells die by AICD while CD8+ T cells undergo apoptosis in the absence of activating signals, i.e. die by 'neglect' has been well established (Lopes *et al*. 1995). During other protozoan infections, however, the mechanism of T cell death appears less clear. After peroral infection of susceptible mice with *T. gondii*, Peyer's patch CD4+ and CD8+ T cells also die via Fas-dependent apoptosis thus resembling AICD (Liesenfeld *et al*. 1997). In contrast, CD4+ splenocytes from mice after i.p. infection with *T. gondii* undergo apoptosis without reactivation *in vitro* thereby leading to unresponsiveness to antigenic or mitogenic signals (Khan *et al*. 1996). In addition, this is at least partially restored by exogenous IL-2, a feature that resembles cell death due to 'neglect' (Nelson and Willerford, 1998). This opens up the possibility that the form of lymphocyte death after parasitic infection also depends on the route of infection and microenvironmental factors. The results, nevertheless, clearly show that both mechanisms of cell death contribute to lymphocyte apoptosis during protozoan infections. More recently, B cells were also shown to undergo apoptosis both *in vitro* and *in vivo* following infection with *T. cruzi* (Zuniga *et al*. 2002) or *P. chabaudi chabaudi* AS (Helmby *et al*. 2000) and this may contribute to defective immunity in response to these protozoans.

Parasite-triggered apoptosis of immune cells not only diminishes the number of specific T and B cells, thereby, abolishing anti-parasitic immune responses but also modulates the reactivity of non-apoptotic cells. Uptake of apoptotic cells displaying a number of 'eat-me' signals including PS or certain carbohydrate moities renders macrophages anti-inflammatory (Savill and Fadock, 2000). This is achieved by inducing the autocrine or paracrine secretion of TGFβ1, PGE_2 and by inhibiting the production of TNFα. Ingestion of apoptotic *L. major*-infected PMN by macrophages indeed leads to considerable release of TGFβ1 but not TNFα, thereby, silencing phagocytes and sustaining parasite multiplication (Van Zandbergen *et al*. 2004). Likewise, parasite-induced AICD in Chagas' disease facilitates replication of *T. cruzi* in macrophages that have ingested apoptotic T cells and this is at least partially due to the anti-inflammatory activity of TGFβ1 and PGE_2 and a reduced NO production (Nunes *et al*. 1998; Freire-de-Lima *et al*. 2000). One might, therefore, speculate that deficiencies in the Fas/Fas-ligand-mediated cell death lead to increased resistance against *T. cruzi*. Surprisingly, however, both Fas- and FasL-deficient mice are even more susceptible to *T. cruzi* parasitaemia since infection leads to a Th2-dominated T cell response with increased IL-4 and IL-10 secretion (Boyer *et al*. 1983; Lopes *et al*. 1999). This example provides clear evidence that parasite-triggered cell death fulfils both 'pro-parasitic' and 'pro-host immunity' functions at least in the course of Chagas' disease probably contributing to a stable parasite-host interaction.

There are also situations in which protozoan parasites trigger an overwhelming apoptosis of host cells thereby abrogating efficient anti-parasitic effector functions, inducing severe host tissue damage and finally resulting in the host's death. For example, infections of mice with a highly virulent strain of *T. gondii* lead to overproduction of Th1-type cytokines but deficient iNOS expression and destruction of spleen tissue due to apoptosis (Mordue *et al.* 2001, Gavrilescu and Denkers, 2001). Beside parasite characteristics, the host genotype also plays a role in such exaggerated depletion of lymphocytes since high level apoptosis of Peyer's patch T cells occurs only in mice susceptible to severe intestinal pathology and death after peroral *T. gondii* infection (Liesenfeld *et al.* 1997). Likewise, after infection with *P. coatneyi*, susceptibility of different species of *Macaca* monkeys also correlated with lymphocytopenia due to extensive T lymphocyte apoptosis (Matsumoto *et al.* 2000). Increased apoptosis may also contribute to massive depletion of lymphocytes during progression of East Coast fever. After an initial phase of lymphocyte proliferation due to the *Theileria*-imposed transformation of leukocytes (see above; Dobbelaere and Heussler, 1999) and parasite-induced expansion of uninfected lymphocytes (Goddeeris and Morrison, 1987), a severe loss of lymphoid tissue due to lymphocytolysis usually occurs (Morrison *et al.* 1981). Whereas the mechanisms of this T cell death is still unknown, it is tempting to speculate that it results from Fas/FasL-dependent AICD and might favour survival of *Theileria*-parastized lymphocytes due to their resistance against Fas-induced apoptosis (Küenzi *et al.* 2003). In conclusion, T lymphocyte apoptosis can, on one hand favour a stable parasite–host coexistence, on the other hand it can also lead to pathological outcomes of protozoan infections. Due to the diverse and possibly also conflicting functions of parasite-triggered host cell apoptosis, it is thus vital to define exactly the impact of apoptosis on parasite development and also on host immunity before its exploitation can be envisaged to combat protozoan diseases. Such exploitation also requires detailed knowledge about the mechanisms by which protozoa parasites modulate host cell apoptosis.

SIGNALLING PATHWAYS REGULATING APOPTOSIS

Due to its crucial roles during development, tissue homeostasis, shaping of the immune system and as an innate and adaptive effector mechanism against pathogens, apoptosis is tightly controlled at both transcriptional and post-transcriptional levels. In mammalian cells, it is initiated and transduced via three major pathways, namely the intrinsic or mitochondrial pathway, the extrinsic or death receptor pathway and the perforin/granzyme pathway (Fig. 1). During recent years, a variety of additional apoptosis-inducing pathways have been described indicating that death signalling appears to be more complex than was originally proposed (Jäättelä and Tschopp, 2003). This includes apoptosis initiated by ligation of Toll-like receptors during the interaction of macrophages and bacterial pathogens (Haase *et al.* 2003; Hsu *et al.* 2004). Whereas several protozoans or molecules thereof clearly trigger TLR-dependent signalling, thereby influencing the course of infection, the effect of such binding on apoptotic programmes of host cells is still unknown. Furthermore, apoptotic cell death can also be triggered by the release of lysosomal enzymes or by ER-related mechanisms, e.g. uncontrolled release of calcium (Ferri and Kroemer, 2001). The impact of infection with protozoans on these cell death mechanisms again remains to be established.

The classical mitochondrial pathway is activated when cells encounter stress signals including DNA damage, growth factor withdrawal, nutrient starvation or toxins (Fig. 1). Importantly, infection by intracellular parasites may also represent an appropriate stress signal, thereby triggering the mitochondrial pathway unless anti-apoptotic activities of the parasite counterbalance this 'natural' cell response (see above). Therefore, the mitochondrial pathway is thought to be of particular relevance for the interaction of intracellular protozoans with apoptotic cascades of the host. The diverse stress-related stimuli converge via largely unknown pathways at the level of several active BH3-only proteins (Bouillet and Strasser, 2002). These proteins function as cellular damage sensors that inactivate anti-apoptotic members of the Bcl-2 family while activating pro-apoptotic Bax/Bak-like Bcl-2 proteins and inducing mitochondrial outer membrane permeabilization (MOMP). The latter leads to the release of cytochrome c from mitochondria into the cytoplasm, where it induces the formation of a multimeric complex, the so-called apoptosome (Fig. 1; Adams and Cory, 2002). The apoptosome formation then activates the initiator caspase 9 which in turn activates the central effector caspase 3. Caspases constitute the central component of apoptotic pathways (Riedl and Shi, 2004) and lead, due to their proteolytic activity, to such cellular and biochemical changes as observed during apoptosis, e.g. cleavage of regulatory and structural proteins, DNA fragmentation and disintegration of the cell. Caspases are synthesized as inactive zymogens referred to as pro-caspases and are activated by cleavage into active subunits although other mechanisms may also participate (Hengartner, 2000).

It should be stressed that beside cytochrome c, other pro-apoptotic proteins are also released from the mitochondria after induction of MOMP. These

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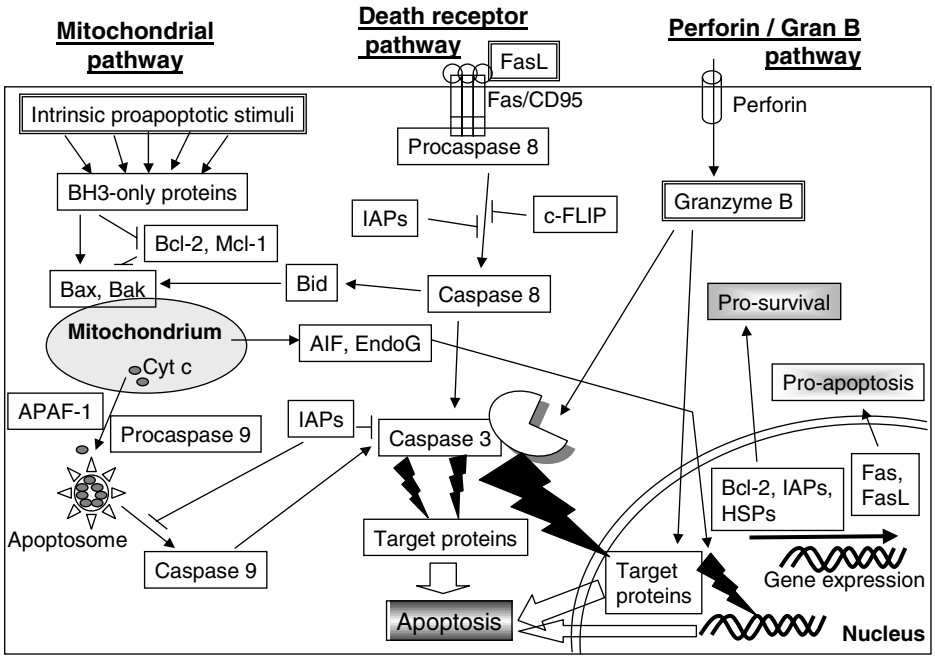

Fig. 1. Signalling and effector cascades regulating apoptosis in mammalian cells. Intrinsic stress-related stimuli, e.g. DNA damage, growth factor deprivation, toxins or infection with intracellular microorganisms can activate the effector caspase 3 via the mitochondrial pathway. Ligation of death receptors, e.g. Fas/CD95 or TNF-R1 either leads to direct activation of caspase 8 and 3 or is amplified via cleavage of Bid and consecutive mitochondrial cytochrome c release. Cytotoxic Natural Killer and T cells can initiate apoptosis by the release of perforin and granzyme B which activate caspase-dependent and independent mechanisms of cell death. All three pathways converge at the level of activated effector caspases, e.g. caspase 3 which cleave various cytosolic and nuclear target proteins then leading to desintegration of the cell. Additional apoptosis-initiating pathways involve proteins which can also operate independently of caspases (e.g. AIF, EndoG). Apoptosis is tightly regulated at both transcriptional and posttranscriptional levels by the expression and activity of proapoptotic (e.g. Fas, FasL, Bax, Bak) and antiapoptotic proteins (e.g. IAPs, HSPs, Bcl-2, c-FLIP). See text for further details.

include the apoptosis-inducing factor (AIF) and the endonuclease G (EndoG) which both translocate into the nucleus and induce DNA fragmentation independently of caspases (Fig. 1). In addition, non-caspase proteases, e.g. cathepsins (Chwieralski, Welte and Buhling, 2006) or calpains (Wang, 2000) also trigger apoptotic cell death and this can occur dependently or independently of caspases. These results show that at least certain forms of apoptosis can be induced without the need of activated caspases (Jäättelä *et al*. 2003; Hong, Dawson and Dawson, 2004).

The second major apoptosis pathway is activated after ligation of so-called death receptors, namely Fas/CD95, tumor necrosis factor-receptor 1 (TNF-R1), or certain TRAIL-Rs (Fig. 1; Ashkenazi and Dixit, 1999). It plays crucial roles in T and B cell development as well as during the course of an immune response and is, therefore, of particular importance for the course of parasitic infections (Dockrell, 2003). Ligation of death receptors leads to the formation of a multimeric complex, i.e. the death-inducing signalling complex (DISC) in which the initiator caspase 8 is proteolytically activated. Owing to the high identity and similar substrate specificity, caspase 10 may also serve as an initiator caspase of the death receptor pathway (Salvesen and Abrams, 2004). Active caspase 8 or 10 in turn activate caspase 3 which is responsible for the execution of apoptosis as described above. In addition, caspase 8 can also indirectly activate caspase 3 via cleavage of the Bid protein (Fig. 1; Scaffidi *et al*. 1999). Truncated Bid is an additional death signal that triggers mitochondrial apoptogenic functions by inducing the release of cytochrome c. Thus, Bid interconnects the extrinsic and intrinsic apoptotic pathways, thereby amplifying death receptor signalling in certain cells or under certain conditions.

The third avenue to death is employed by cytotoxic lymphocytes, i.e. T lymphocytes and Natural Killer (NK) cells to eliminate malignant or pathogen-infected cells (Lieberman, 2003). Furthermore, recent evidence suggests that this pathway plays a major role in immunological homeostasis (Trambas and Griffiths, 2003). After cell–cell interaction, serine proteases known as granzymes are exocytosed and enter the target cell with the help of membrane-perturbing proteins, e.g. perforin (Fig. 1). Granzymes then induce apoptosis by caspase-dependent and -independent mechanisms including direct proteolytic activation of caspases, cleavage of Bid

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and consecutive activation of the mitochondrial pathway as well as induction of DNA fragmentation (Lieberman, 2003).

Due to their vital roles, the initiation and transduction of apoptotic cascades are strictly regulated and can be counteracted by multiple pro-survival signals (Fig. 1). Such molecules not only suppress physiological apoptotic cell death, but also represent possible targets for anti-apoptotic activities of protozoan parasites (see below). Cellular survival-promoting proteins include members of the Bcl-2 and the death effector domain (DED) families, inhibitors of apoptosis (IAPs), TNF-R-associated factors (TRAFs) and heat shock proteins (HSPs) (Ekert, Silke and Vaux, 1999; Beere and Green, 2001; Tibbetts, Zheng and Lenardo, 2003; Cory, Huang and Adams, 2003).

MECHANISMS BY WHICH PROTOZOANS MODULATE THE HOST'S APOPTOTIC PROGRAMMES

In addition to the gathering of detailed knowledge on the impact of parasite-mediated modulation of host cell apoptosis on the course of infection, unravelling the underlying cellular and molecular mechanisms is also of major importance. With the rapidly increasing understanding of the regulation of apoptosis, much progress has also been made in elucidating the mechanisms by which protozoan parasites manipulate the host's apoptotic programme. Consistent with the diverse life styles of different protozoans and their heterogeneous effects on apoptosis of the host cells, multiple strategies have been specifically evolved to favour the parasite's requirements. Other parasite-mediated effects on apoptosis and their underlying mechanisms, for example AICD via Fas ligation may rather represent the 'normal' response of the host in order to turn lymphocytes off and to avoid immunopathology. Despite this diversity, there are, nevertheless, some common themes which are involved in the alteration of host cell death after parasitic infections (Table 2). A useful classification differentiates between direct and indirect mechanisms. The former requires a direct interaction of the parasite with the manipulated cell of the host, while the latter also affects bystander cells which have not directly encountered the parasite (Lüder and Gross, 2005). Both parasite-derived and host-derived molecules may mediate the alteration of host cell death in most situations; however, there are also examples where direct interferences are exclusively triggered by parasite-derived effectors. Parasite–host interactions involving such parasitic effectors could be of particular relevance, because they provide not only a more direct mean to combat parasitic infections but possibly also lead to the identification of previously unrecognized mechanisms and/or regulators of apoptosis.

Modulation of apoptosis by contact-dependent mechanisms

E. histolytica induces apoptosis in target cells by a contact-dependent mechanism that is mediated via the interaction of the parasitic Gal/GalNAc adherence lectin (Table 2; Huston *et al.* 2000) and still unknown surface molecules with terminal galactose residues (Li, Becker and Stanley, 1989). A strong increase in the intracellular Ca^{2+} concentration of the target cell seems to be involved but is certainly not the only factor in the induction of apoptosis (Ravdin *et al.* 1988). Importantly, caspase 3 is clearly activated which occurs independently of the upstream initiator caspases 8 and 9 (Huston *et al.* 2000). Whether other initiator caspases, e.g. caspase 10 are involved or whether caspase 3 is directly activated in a fashion similar to that employed by granzyme B remains to be resolved. Recently, increased expression and activation of several protein kinases were implicated in apoptosis induced by *E. histolytica* (Rawal, Majumdar and Vohra, 2005; Sim *et al.* 2005) and reactive oxygen species-mediated activation of ERK1/2 appears to be of particular relevance at least in neutrophils (Sim *et al.* 2005). This came as a surprise since ERKs are known better as signals that support cell survival rather than apoptosis. However, ERK activation appears to be particularly important in reactive oxygen species-mediated apoptosis (Seo *et al.* 2001) and it will be interesting to see whether ERK activation also plays a crucial role in *Entamoeba*-induced apoptosis in cell types other than neutrophils.

As outlined above, induction of apoptosis in endothelial cells may play a role in the pathophysiology of cerebral malaria. Sequestration of parasite-infected erythrocytes and subsequent blockade of microvessels is crucial for this severe complication and involves the interaction of different *P. falciparum* or erythrocyte molecules exposed on the red blood cell surface with endothelial receptors (Eda and Sherman, 2002). Importantly, direct contact between parasitized erythrocytes and endothelial cells has recently been shown to lead to activation of the caspase cascade and to the induction of apoptosis in a redox-dependent manner (Table 2; Pino *et al.* 2003*a*, *b*).

Cytokine secretion and modulation of apoptosis

Growth factors and cytokines are prominent regulators of apoptosis and are also exploited by protozoans to modulate apoptosis in the host. They are involved in both activation and inhibition of host cell death after parasitic infection. Obviously, modulation of apoptosis via the induction of cytokine secretion represents an indirect mechanism affecting both parasite-positive cells as well as parasite-negative bystander cells. After infection of mice with

Table 2. Direct and indirect mechanisms of protozoan parasites to alter apoptosis in their hosts

Mechanism	Type of interaction	Effect	Parasite species	References
Contact-dependent				
Parasite-target cell interaction	Direct	Target cell apoptosis	*E. histolytica*	Huston *et al.* 2000; Sim *et al.* 2005
Cytoadherence of infected erythrocytes	Direct	Apoptosis in endothelial cells	*P. falciparum*	Pino *et al.* 2003*a*, 2003*b*
Cytokine-mediated				
IFN-γ, proinflammatory cytokines	Indirect	AICD/splenocyte apoptosis	*T. gondii*	Liesenfeld *et al.* 1997; Gavrilescu *et al.* 2001; Mordue *et al.* 2001
			T. cruzi	Martins *et al.* 1999
GM-CSF, G-CSF	Indirect	Neutrophil survival	*T. gondii*	Channon *et al.* 2002
Increased gene expression of pro-apoptotic host molecules				
iNOS	Indirect	Splenocyte apoptosis	*T. cruzi*	Martins *et al.* 1999
Fas, Fas ligand	Indirect	AICD, apoptosis in non-T cells	*P. falciparum*	Kern *et al.* 2000; Toure-Balde *et al.* 2000
			T. gondii	Liesenfeld *et al.* 1997; Hu *et al.* 1999
			Leishmania spp.	Desbarats *et al.* 2000; Eidsmo *et al.* 2005
			T. cruzi	Martins *et al.* 1999
			C. parvum	Chen *et al.* 1999
TNF-R1	Indirect	Splenocyte apoptosis	*L. major*	Kanaly *et al.* 1999
			T. gondii	Gavrilescu *et al.* 2001
Increased gene expression of anti-apoptotic molecules				
Anti-apoptotic Bcl-2 proteins	Direct	Blockade of host cell suicide	*T. gondii*	Goebel *et al.* 2001; Molestina *et al.* 2003
			T. cruzi	Chuenkova *et al.* 2000; Aoki *et al.* 2004
			C. parvum	Mele *et al.* 2004
	Indirect	Neutrophil/macrophage survival	*T. gondii*	Channon *et al.* 2002; Orlofsky *et al.* 2002
IAPs	Direct	Blockade of host cell suicide	*T. gondii*	Molestina *et al.* 2003
	Direct	Reduced Fas-induced apoptosis	*T. parva*	Küenzi *et al.* 2003
HSP65	Indirect	Macrophage survival	*T. gondii*	Hisaeda *et al.* 1997
			T. cruzi	Sakai *et al.* 1999
			L. major	Ishikawa *et al.* 2000
c-FLIP	Direct	Reduced Fas-induced apoptosis	*T. parva*	Küenzi *et al.* 2003
c-myc	Direct (?)	Reduced caspase 9 activation	*T. parva*	Dessauge *et al.* 2005*b*
Interference with apoptotic cascades				
Stabilization of c-FLIP	Direct	Inhibition of caspase 8 activation	*T. cruzi*	Hashimoto *et al.* 2005
Activation of Akt/PKB	Direct (?)	Inhibition of apoptosis	*T. cruzi*	Chuenkova *et al.* 2001
Activation of NF-κB	Direct	Inhibition of apoptosis	*T. parva*	Heussler *et al.* 1999, 2002
			T. gondii	Payne *et al.* 2003; Molestina *et al.* 2005
Activation of JNK	Direct	Inhibition of apoptois	*T. parva*	Lizundia *et al.* 2005
Activation of casein kinase II	Direct	Inhibition of caspase 3 activity	*T. parva*	Dessauge *et al.* 2005*a*
Decreased JNK activation	Direct (?)	Block of UV-induced apoptosis	*T. gondii*	Carmen *et al.* 2006
Inhibition of caspase activation	Direct	Reduced caspase 3 activity	*T. gondii*	Keller *et al.* 2006
Decrease in PARP protein levels	Direct (?)	Inhibition of apoptosis	*T. gondii*	Goebel *et al.* 2001

virulent *T. gondii*, AICD correlates with high levels of proinflammatory cytokines including IFNγ (Table 2; Gavrilescu and Denkers, 2001; Mordue *et al.* 2001). Recent findings that IFNγ is required for AICD and regulates the expression of Fas/FasL as well as caspases including caspase 8 (Refaeli *et al.* 2002) clearly argues for a crucial involvement of the cytokine in parasite-mediated T cell apoptosis. IFNγ has indeed been shown to induce expression of FasL and/or Fas in T cells after infection with *T. cruzi* (Martins *et al.* 1999) and with *T. gondii* at least under conditions of severe immunopathology (Liesenfeld *et al.* 1997; Gavrilescu and Denkers, 2001). In addition, IFNγ also triggers splenocyte apoptosis by upregulation of the inducible nitric oxide synthase (iNOS) and subsequent NO production in *T. cruzi*-infected mice (Martins *et al.* 1999). It thus appears plausible that induction of an inflammatory response after infection with certain protozoan parasites also leads to negative feedback mechanisms including AICD that limit the immune response in order to avoid severe immunopathology.

Cytokines were also shown to promote cell survival after infection with protozoan parasites. After infection of fibroblasts, *T. gondii* induces secretion of granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage CSF (GM-CSF) and this rescues human neutrophils from apoptosis (Table 2; Channon *et al.* 2002). It has, therefore, been hypothesized that the sustained neutrophil survival may contribute to the robust pro-inflammatory response elicited by *T. gondii*. Importantly, infection of mice with *T. gondii* increases neutrophil and macrophage numbers in the peritoneal exsudate and triggers expression of the GM-CSF-regulated anti-apoptotic Bcl-2 family member A1/Bfl-1 (Orlofsky *et al.* 1999). This provides clear evidence that parasite-triggered GM-CSF may also facilitate survival of inflammatory cells *in vivo* after infection with *T. gondii*.

Up-regulation of pro- and anti-apoptotic host molecule gene expression by parasitic protozoa

Due to the vital roles of apoptosis, expression of pro- and anti-apoptotic regulators are tightly controlled. A variety of protozoan parasites exploit these regulators by modulating their expression in the host. AICD, for example, is induced in a Fas/FasL-dependent manner and several protozoans including *P. falciparum* (Kern *et al.* 2000; Toure-Balde *et al.* 2000), *T. gondii* (Liesenfeld *et al.* 1997; Hu *et al.* 1999), *Leishmania* spp. (Desbarats *et al.* 2000; Eidsmo *et al.* 2002, 2005) and *T. cruzi* (Martins *et al.* 1999) indeed promote expression of these molecules (Table 2). In addition, Fas is also up-regulated after parasitic infection of cells others than T cells (Chen *et al.* 1999; Hu *et al.* 1999; Eidsmo *et al.* 2005). Beside Fas and FasL, up-regulation of the TNF-R1 (p55) may also participate in the induction of T cell death and the restriction of the inflammatory response at least to *L. major* and *T. gondii* (Table 2; Kanaly *et al.* 1999; Gavrilescu and Denkers, 2001).

In order to inhibit apoptosis, protozoan parasites exploit anti-apoptotic molecules of the host cell by triggering their expression after infection. Such molecules include members of three protein families with well known anti-apoptotic functions, i.e. certain Bcl-2 proteins, IAP's and HSPs as well as FLIP and the protooncogene c-myc (Table 2). Although the exact mechanisms are still a matter of debate, anti-apoptotic Bcl-2 family members, i.e. Bcl-2, Bcl-xL, Mcl-1, A1/Bfl-1 and others counteract the pro-apoptotic Bax and Bak executioners of mitochondrial cytochrome c release (Cory *et al.* 2003). Consequently, up-regulation of Mcl-1 (Goebel *et al.* 2001; Channon *et al.* 2002) and A1/Bfl-1 (Orlofsky *et al.* 2002; Molestina *et al.* 2003) after infection with *T. gondii* might indeed participate in the block of cytochrome c release in infected host cells (Goebel *et al.* 2001). This block is strictly confined to parasite-positive host cells thus indicating a direct interaction of *T. gondii* and the host cell (Goebel *et al.* 1999, 2001). Since Mcl-1 and A1 are also up-regulated in uninfected bystander cells (Orlofsky *et al.* 1999; Channon *et al.* 2002) additional factors are likely to be involved in the direct interference of *T. gondii* with cytochrome c release in parasite-positive cells. Up-regulation of the anti-apoptotic Bcl-2 has also been described after infection of cardiomyocytes and neuronal cells with *T. cruzi* and correlates with prevention of growth factor deprivation-mediated apoptosis (Chuenkova and Pereira, 2000; Aoki *et al.* 2004). Likewise, transient inhibition of apoptosis by *C. parvum* might also involve increased expression of Bcl-2 (Mele *et al.* 2004). However, the exact contribution of parasite-mediated up-regulation of anti-apoptotic Bcl-2 proteins in the inhibition of apoptosis remains to be established. Furthermore, the effect of parasitic infections on BH3-only proteins, i.e. crucial regulators of anti-apoptotic Bcl-2 family members also needs clarification.

Another prominent group of proteins with well known anti-apoptotic functions are the IAPs which inhibit caspases by direct interaction (Deveraux *et al.* 1997; Ekert *et al.* 1999). While homologues of IAPs are known to inhibit apoptosis after infection with several viruses, much less is known on the effect of protozoan parasites on these molecules. Gene array and RT-PCR analyses performed by Molestina *et al.* (2003) revealed that IAP-2 and neuronal (N) IAP-1 are up-regulated in murine fibroblasts infected with *T. gondii* (Table 2). This raises the possibility that *T. gondii*, besides increasing expression of Bcl-2 family members, also up-regulates IAPs, thereby directly interfering with caspase

activation. However, the functional consequence of this finding awaits clarification. *T. parva* obviously employs XIAP and c-IAP 1 to render T cells resistant to Fas-induced apoptosis, since elimination of the parasite leads to the rapid decrease of these IAPs and to a concomitant increase in Fas-mediated apoptosis (Küenzi *et al*. 2003). Strikingly, expression of both IAPs and anti-apoptotic Bcl-2 family members is regulated in a NF-κB-dependent manner suggesting a common mechanism in triggering their gene expression after parasitic infection. Activation of the transcription factor NF-κB has indeed been described after infection with a variety of protozoans including *T. parva* (Heussler *et al*. 1999), *C. parvum* (McCole *et al*. 2000; Chen *et al*. 2001), *T. gondii* (Payne *et al*. 2003) and *L. major* (Artis *et al*. 2003) and may thus represent an important common mechanism to prevent host cell apoptosis.

In addition to IAPs, expression of cellular FLIP (c-FLIP) was also found to be up-regulated after infection of T lymphocytes with *T. parva* (Küenzi *et al*. 2003). C-FLIP is a short-lived inhibitor of the processing of caspase 8 at the death inducing signalling complex (DISC) and might therefore also be involved in the resistance of *Theileria*-infected cells against death receptor-induced signalling (Table 2). Whereas c-FLIP may be required to block death-receptor-induced apoptosis, increased transcription of the protooncogene c-myc reduces – possibly via the induction of anti-apoptotic Mcl-1 – the activation of caspase 9, i.e. the mitochondrial pathway of apoptosis in *Theileria*-infected B lymphocytes (Table 2; Dessauge *et al*. 2005*b*). Different molecules may thus confer resistance of *Theileria*-infected host cells against distinct pro-apoptotic stimuli. Alternatively, increased expression of IAPs, c-FLIP and c-myc after infection with *Theileria* could also represent redundant safeguard mechanisms to ensure host cell and parasite survival.

HSPs are chaperones that are up-regulated in cells encountering several stress signals and may protect from apoptosis (Beere and Green, 2001). Infections with protozoan parasites including *T. cruzi* (Sakai *et al*. 1999), certain strains of *T. gondii* (Hisaeda *et al*. 1997) and *L. major* (Ishikawa *et al*. 2000) were indeed shown to trigger HSP65 expression in inflammatory macrophages (Table 2). Interestingly, HSP65 diminished apoptosis in macrophages and was associated with increased resistance to the parasite. However, the molecular basis for the apoptosis-blocking effect of parasite-induced HSP65 remains unknown. HSP27, HSP70 and HSP90 block the mitochondrial apoptotic pathway by interfering with the apoptosome formation (Beere and Green, 2001; Bruey *et al*. 2000), but this has not been established for HSP65. It will thus be interesting to unravel, whether parasite-induced HSP65 fulfils a similar effect as previously described for the other HSPs, or inhibits apoptosis by different means.

Direct interference of protozoans with apoptotic cascades

There is increasing evidence suggesting that protozoan parasites also directly modulate apoptotic cascades of the host. This may either be achieved by interaction of parasite or host-derived molecules with apoptotic cascades or regulatory elements thereof. For example, *T. cruzi* was recently shown to exploit c-FLIP to inhibit Fas-mediated apoptosis in parasite-infected cells *in vitro* and *in vivo* (Table 2; Hashimoto *et al*. 2005). Infection with *T. cruzi* considerably stabilizes c-FLIP, thereby, inhibiting death receptor-induced caspase activation (Hashimoto *et al*. 2005). In addition, the *T. cruzi* trans-sialidase potently activates the phosphatidylinositol 3-kinase/Akt (PI 3-k/Akt) pathway, thereby blocking growth factor deprivation-mediated apoptosis in Schwann cells, the primary target cells of the parasite in the peripheral nervous system (Table 2; Chuenkova *et al*. 2001). Akt/PKB is able to directly inhibit apoptosis via different mechanisms, e.g. the phosphorylation and inhibition of the pro-apoptotic Bcl-2 family protein Bad (Song, Ouyang and Bao, 2005). This suggests that *T. cruzi* might inhibit the mitochondrial apoptotic pathway by interfering with pro-apoptotic host cell pathways via the activation of Akt/PKB. However, due to the multiple functions of the PI 3-k/Akt pathway, such an effect of the *T. cruzi* trans-sialidase remains to be established. During infection with the apicomplexan *T. parva*, Akt/PKB is involved in the transformation of T cells, but not in the parasite-mediated inhibition of apoptosis (Heussler *et al*. 2001*b*; see also Heussler, Sturm and Langsley, in this supplement). In contrast to Akt/PKB, constitutive activation of NF-κB by intracellular *Theileria* has been shown to be crucial to protect parasite-transformed T cells from apoptosis (Heussler *et al*. 1999). Interestingly, activation of NF-κB is accomplished by the recruitment and activation of the components of the IκB kinase (IKK) complex to the parasite surface, which then leads to the degradation of IκBα and consequently to the nuclear import of NF-κB (Heussler *et al*. 2002). More recently, activation of the c-Jun N-terminal kinase (JNK) has also been implicated in the anti-apoptotic activity of *Theileria* (Lizundia *et al*. 2005). Furthermore, inhibition of the casein kinase II (CK2) leads to an increased caspase 3 activity in *Theileria*-infected B cells (Dessauge, Lizundia and Langsley, 2005*a*). Whereas the exact contribution of these pathways to the inhibition of host cell apoptosis remains to be established, it suggests that *Theileria* employs multiple mechanisms to avoid the death of its host cell (for further details see Heussler, Sturm and Langsley, in this supplement).

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Direct interferences with different host cell signalling cascades obviously also renders host cells of *T. gondii* resistant against apoptosis. In contrast to *T. parva*, a parasite-derived kinase activity rather than host IKK has been described to phosphorylate IκBα and to activate NF-κB (Molestina and Sinai, 2005; Payne *et al*. 2003). It should be mentioned that the effect of intracellular *T. gondii* on the NF-κB pathway has not yet been completely resolved and further experiments are required to unequivocally unravel its role during the inhibition of host cell apoptosis (Lüder and Gross, 2005). During UV light-induced apoptosis, *T. gondii* inhibits activation of JNK and leads to the degradation of Bax and Bad, i.e. pro-apoptotic Bcl-2 family members (Carmen, Hardi and Sinai, 2006). Due to the crucial role of JNK during UV-induced apoptosis and of Bax in the induction of MOMP, parasite interferences with their expression or activities are potentially important mechanisms to inhibit apoptosis. It will be of particular interest, whether these mechanisms also confer resistance against other pro-apoptotic stimuli than UV.

Recently, we described the direct inhibition of the cytochrome c-mediated activation of the caspase 3 by *T. gondii* (Table 2; Keller *et al*. 2006), which potentially also plays a crucial role in the inhibition of host cell apoptosis. Importantly, since such inhibition was observed in a cell-free *in vitro* system, this provides the first direct evidence for an interaction of *T. gondii* with a caspase cascade that operates independently of a decreased release of cytochrome c from the host cell mitochondria (Goebel *et al*. 2001) and functional transcription and translation machineries of the host cell (Goebel *et al*. 2001; Molestina *et al*. 2003). However, the functional relevance of this novel interaction within the infected cell remains to be established.

Goebel *et al*. (2001) showed that the blockade of host cell apoptosis imposed by *T. gondii* correlates with considerably diminished protein levels, but not mRNA levels of the poly(ADP-ribose) polymerase (PARP; Table 2). This enzyme is well known as a target of effector caspase activity during apoptosis, but there is now convincing evidence that PARP – in addition to other functions – also serves as an important pro-apoptotic factor under conditions of excessive PARP activity (Simbulan-Rosenthal *et al*. 1998). Activation of PARP leads to the depletion of NAD+ and ATP, mitochondrial outer membrane permeabilization (MOMP), release of apoptosis-inducing factor (AIF) and caspase-independent cell death (Hong *et al*. 2004). Due to the severely decreased PARP protein levels after infection with *T. gondii*, it may thus be hypothesized that this contributes to the parasite-mediated inhibition of host cell apoptosis at least under certain conditions. However, since *T. gondii* also inhibits caspase 9 and 3 activation, additional mechanisms other than down-regulation of PARP are clearly also involved in the parasite-mediated inhibition of apoptosis (see above).

CONCLUDING REMARKS

Undoubtedly, pro- and anti-apoptotic activities of protozoans are fascinating examples of the versatile mechanisms by which parasites exploit essential cellular functions of the host. More importantly, however, these activities have major impact on the outcome of infection and may favour a stable parasite-host interaction or parasite- and immune-mediated pathology. Apoptosis and its modulation by protozoan parasites thus potentially represent a novel target to treat life-threatening diseases. Such a scenario is feasible, since manipulation of apoptosis for the therapy of diseases that involve either deficient or exaggerated apoptosis, i.e. certain types of cancer and neurodegenerative diseases, respectively, may be exploited in the near future (Nicholson, 2000; Cummings *et al*. 2004). Some of the examples of parasite interactions with the host's apoptotic programme discussed above also provide clear evidence of a still unknown level of complexity in the interaction of microorganisms with the host's apoptotic cell death. Thus, whether treatment of parasitic diseases via the manipulation of the pro- and anti-apoptotic activities of protozoans will ever become reality is a matter of speculation. Detailed knowledge of the underlying molecular and cellular mechanisms is clearly one crucial prerequisite before the aim of novel therapies can be accomplished. Despite the considerable progress being made in defining the pathways by which protozoans modulate apoptosis, further efforts are clearly needed to unravel these pathways more precisely. Particularly, we are just beginning to characterize those molecules which mediate the pro- and anti-apoptotic activities of protozoan parasites and this remains a major task for future work. Nevertheless, with our rapidly increasing knowledge on these parasite-host interactions, we are well on the way at least to determine whether exploitation of pro-and anti-apoptotic properties will be feasible to combat parasitic diseases.

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