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Apoptosis and Its Modulation During Infection with *Toxoplasma gondii*: Molecular Mechanisms and Role in Pathogenesis

C. G. K. Lüder (✉) · U. Gross

Institute of Medical Microbiology, Georg-August-University, Kreuzbergring 57, 37075 Göttingen, Germany
clueder@gwdg.de

Abstract Infection with the obligate intracellular protozoan *Toxoplasma gondii* leads to lifelong persistence of the parasite in its mammalian hosts including humans. Apoptosis plays crucial roles in the interaction between the host and the parasite. This includes innate and adaptive defense mechanisms to restrict intracellular parasite replication as well as regulatory functions to modulate the host's immune response. Not surprisingly, however, *T. gondii* also extensively modifies apoptosis of its own host cell or of uninfected bystander cells. After infection, apoptosis is triggered in T lymphocytes and other leukocytes, thereby leading to suppressed immune responses to the parasite. T cell apoptosis may be largely mediated by Fas engagement but also occurs independently of Fas under certain conditions. Depending on the magnitude of T cell apoptosis, it is either associated with unrestricted parasite replication and severe pathology or facilitates a stable parasite-host-interaction. However, *T. gondii* has also evolved strategies to inhibit host cell apoptosis. Apoptosis is blocked by indirect mechanisms in uninfected bystander cells, thereby modulating the inflammatory response to the parasite. In contrast, inhibition of apoptosis in infected host cells by direct interference with apoptosis-signaling cascades is thought to facilitate

the intracellular development of *T. gondii*. Blockade of apoptosis by intracellular parasites may be achieved by different means including interference with the caspase cascade, increased expression of antiapoptotic molecules by infected host cells, and a decreased activity of the poly(ADP-ribose) polymerase. The intriguing dual activity of *T. gondii* to both promote and inhibit apoptosis requires a tight regulation to promote a stable parasite host-interaction and establishment of persistent toxoplasmosis.

1 Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects a broad range of warm-blooded animals including approximately 30% of the human population worldwide (Tenter et al. 2000). Infection is orally acquired by ingestion of infectious sporozoites that have been released by parasitized cats via the feces or by ingestion of persisting parasites contained in raw or undercooked meat from intermediate hosts. Furthermore, transmission can occur vertically by transplacental transmission to the offspring (see below). During primary infection, disseminating parasites, the so-called tachyzoites, are able to actively invade and rapidly replicate in nearly any cell type investigated so far. In the face of an adequate immune response, these parasites differentiate into metabolically dormant cyst-forming bradyzoites that are able to persist predominantly in neural and muscular tissue. Infection of immunocompetent individuals is mostly asymptomatic or leads to mild symptoms only but gives rise to chronic infection that persists throughout the host's life. However, *T. gondii* is a major opportunistic pathogen of newborns from recently infected mothers (Petersen et al. 2001) and of immunocompromised patients, i.e. those with AIDS or under immunosuppressive therapy (Ammassari et al. 1996). In these individuals, the premature or suppressed immune system is not able to control parasite multiplication, eventually leading to life-threatening disease.

The importance of T cell-mediated immunity for antiparasitic activity has been supported by experimental infections in mice showing that $CD8^+$ and $CD4^+$ T lymphocytes are required to avoid unrestricted parasite multiplication (Suzuki and Remington 1988; Gazzinelli et al. 1992). Whereas $CD8^+$ T cells appear to represent the most important effector cells against *T. gondii*, $CD4^+$ T cells rather fulfill important regulatory functions (Denkers and Gazzinelli 1998). During the early phase of infection, natural killer (NK) cells may fulfill T cell-independent effector functions against the parasite (Sher et al. 1993). Production of IFN-γ

and additional proinflammatory cytokines, e.g. TNF-α, activate infected cells to exert antiparasitic activity and are the major mediators of resistance against *T. gondii* (Suzuki et al. 1988; Sibley et al. 1991). Furthermore, lysis of infected target cells by $CD8^+$, $CD4^+$, and NK cells may also contribute to parasite control at least under certain conditions (Denkers et al. 1997; Hu et al. 1999; Curiel et al. 1993). Despite the induction of these potentially effective immune responses against *T. gondii*, the host is nevertheless unable to clear the infection.

A variety of factors have been described that may contribute to the parasite's ability to establish and maintain a persistent infection in its immunocompetent host. Accumulating evidence indicates that this also includes alterations of apoptosis in distinct host cell populations (Lüder et al. 2001; Heussler et al. 2001). This is not surprising because apoptosis is known to play a critical role in the regulation of the immune response (Opferman and Korsmeyer 2003), as an effector mechanism of NK cells and cytotoxic T lymphocytes (CTL) to eliminate infected target cells (Lieberman 2003), and as an innate response of cells after infection by intracellular pathogens (Williams 1994). More interestingly, *T. gondii* both promotes and inhibits apoptosis. Inhibition of host cell apoptosis may allow undisturbed intracellular development, thereby facilitating parasite survival. Increased apoptosis of immune cells after infection, on the other hand, is thought to partially downregulate effective immune responses against *T. gondii* leading to immune evasion. However, host cell apoptosis and its modulation by the parasite not only benefit *T. gondii*, but are also required to restrict tissue destruction by inflammatory responses, i.e. immunopathology.

Here we review the current knowledge on the molecular mechanisms of the dual activity of *T. gondii* in apoptosis. We also focus on the role of apoptosis and its modulation by the parasite for the course of toxoplasmosis and for the pathogenesis of disease.

2 Infection with *T. gondii* as a Trigger of Apoptosis

Acute infection of both humans and mice with *T. gondii* induces a state of transient immunosuppression as determined by decreased antibody and T lymphocyte responses to homologous and heterologous antigens (Strickland and Sayles 1977; Wing et al. 1983; Luft et al. 1984; Yano et al. 1987). Among other factors, apoptosis of T lymphocytes triggered by *T. gondii* may restrict the immune response to the parasite (Khan et al.

1996; Liesenfeld et al. 1997; Wei et al. 2002). Indeed, high levels of apoptosis in splenocytes have recently been associated with unrestricted parasite multiplication leading to high parasite burdens in various tissues of the host (Mordue et al. 2001; Gavrilescu and Denkers 2001). Cell death within the spleen was not restricted to certain populations but was detected in $CD4^+$ and $CD8^+$ T lymphocytes, B lymphocytes, NK cells, and granulocytes (Gavrilescu and Denkers 2003). It must be stressed, however, that the level of splenocyte apoptosis was markedly determined by the genotype (Grigg et al. 2001) and the virulence of the parasite. This raises the possibility that parasite-triggered extensive apoptosis of leukocytes is not a general phenomenon after infection with *T. gondii* but rather represents a characteristic determinant of the course of toxoplasmosis. Importantly, lymphocyte apoptosis may also influence the local immune response after natural parasite transmission via the gut, because oral infection with *T. gondii* led to apoptosis in Peyer's patch T cells (Liesenfeld et al. 1997). Such cell death again appears to depend on the course of infection, being observed only in inbred mouse strains susceptible to severe disease (McLeod et al. 1996).

Apoptosis of $CD4^+$ T cells was preceded by a state of unresponsiveness to antigenic or mitogenic stimulation (Khan et al. 1996). Because T cell activation markers could be readily detected, this is reminiscent of a similar condition described by Lopes et al. (1995) during experimental Chagas disease, i.e. activation-induced cell death (AICD). This form of apoptosis is initiated by the interaction of Fas and FasL, augmented by IL-2, and is counteracted by Bcl-2 or Bcl-X_L (Van Parijs and Abbas 1996). Infection with *T. gondii* indeed led to the upregulation of Fas expression in Peyer's patch T cells (Liesenfeld et al. 1997) as well as splenocytes and ocular tissue (Hu et al. 1999). Furthermore, induction of apoptosis by *T. gondii* was abolished in mutant mice lacking a functional Fas-FasL system (Liesenfeld et al. 1997; Gavrilescu and Denkers 2003). Expression of Fas as well as Fas-FasL-mediated apoptosis in *T. gondii*-infected mice appear to be regulated by the secretion of proinflammatory cytokines, IL-12 and IFN-γ, and may be counterbalanced by activation of NF-κB_2 (Caamano et al. 2000). These results clearly suggest a crucial role of the interaction of Fas and its ligand in *T. gondii*-triggered apoptosis of T cells.

Recently, human dendritic cells infected with viable, but not nonviable, parasites have been shown to induce T lymphocyte apoptosis in a contact-dependent and Fas-independent fashion (Wei et al. 2002). Beside AICD, T cell death in the absence of Fas ligation (Van Parijs and Abbas 1996) may thus also contribute to T lymphocyte dysfunction during tox-

oplasmosis. Whether such form of apoptosis operates in vivo, however, is yet unknown. It nevertheless raises the possibility that both Fas-dependent and -independent cell death deplete T cells during toxoplasmosis. Further experiments are needed to unravel the relative contribution of these different forms of cell death for the course of infection. Furthermore, its overall impact on the transient immunosuppression during toxoplasmosis also awaits further clarification.

3
Inhibition of Host Cell Apoptosis by *T. gondii*

Besides increased apoptosis of distinct cell populations after infection, *T. gondii* has been shown clearly to also decrease host cell death (Hisaeda et al. 1997; Nash et al. 1998; Goebel et al. 1999, 2001; Channon et al. 2002; Payne et al. 2003). Parasite-mediated resistance against apoptosis was observed in both murine and human cell lines treated with diverse inducers of apoptosis, including CTL-mediated cytotoxicity, irradiation, growth factor withdrawal, TNF-α, and/or several toxic agents (Nash et al. 1998; Goebel et al. 2001; Payne et al. 2003). Furthermore, *T. gondii* also led to decreased apoptosis in primary cells cultured ex vivo after growth factor withdrawal (Hisaeda et al. 1997; Channon et al. 2002). Importantly, inhibition of apoptosis has recently also been shown to occur in vivo after intraperitoneal infection of mice with *T. gondii* (Orlowsky et al. 1999, 2002). This suggests that interference of *T. gondii* with the suicide program of host cells may modify the course of toxoplasmosis.

Three major pathways are known to trigger apoptosis in response to external or internal stimuli: (a) binding of death ligands to their specific cell surface receptors, such as Fas or TNF receptor I (death receptor pathway; Tibbetts et al. 2003), (b) release of cytochrome *c* from the mitochondria into the cytosol induced by irradiation, toxic agents, cellular stress, or growth factor withdrawal (mitochondrial pathway; Green and Reed 1998), and (c) release of perforin and granzymes by NK cells and CTL (granule-mediated cytotoxicity; Lieberman 2003). Although caspase-independent cell death may occur under certain conditions (Lieberman 2003), the apoptosis-regulating pathways mostly converge at the level of activated caspase 3 and other effector caspases, which finally lead to those cellular changes associated with apoptosis (Kaufmann and Hengartner 2001). Because *T. gondii* targets apoptosis induced by a wide range of different stimuli (see above) it might be hypothesized that this is achieved by parasite interference with a component common to all

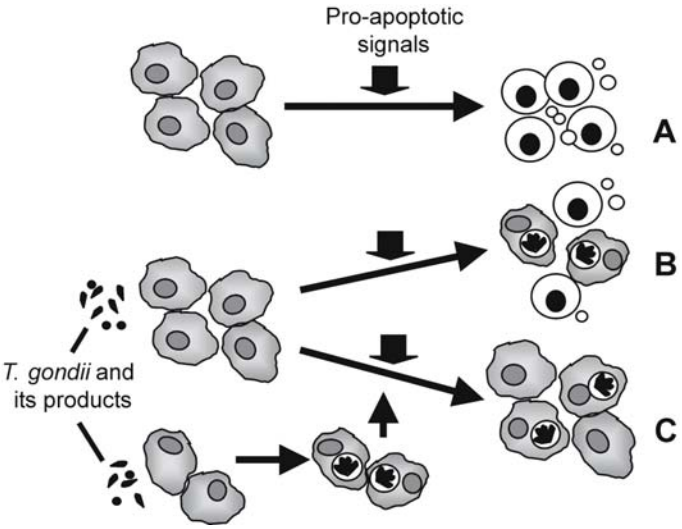

Fig. 1 Different mechanisms of *T. gondii* contribute to inhibition of host cell apoptosis during infection. **A** In the absence of the apoptosis-blocking features of the parasite, proapoptotic signals either related or unrelated to infection induce apoptosis. **B** In the presence of the parasite and its products, proapoptotic signals induce apoptosis in parasite-negative cells only, whereas direct interference of the parasite with signaling in parasite-positive host cells blocks apoptosis. **C** Apoptosis induced by proapoptotic signals may also be inhibited by indirect mechanisms, i.e., production of host-derived antiapoptotic molecules by *Toxoplasma*-infected cells. These molecules in turn block apoptosis in parasite-infected cells and uninfected bystander cells

pathways. To date, however, experimental evidence rather suggests that *T. gondii* inhibits apoptosis of host cells by different mechanisms. Whereas direct inhibition of host cell apoptosis by *T. gondii* is restricted to parasite-positive host cells, indirect mechanisms protect both infected and uninfected host cells against apoptosis (Fig. 1).

3.1 Indirect Mechanisms That Inhibit Apoptosis in Host Cells During Infection

Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage CSF (GM-CSF) secreted by *T. gondii*-infected human fibroblasts increased expression of the antiapoptotic Bcl-2 family member Mcl-1 and abolished apoptosis in neutrophils in vitro (Fig. 1C; Channon et al. 2002). A similar mechanism may also operate in vivo, because the in-

flammatory response to *T. gondii* after intraperitoneal infection of mice is accompanied by increased levels of A1, an antiapoptotic protein similar to Mcl-1 (Orlofsky et al. 1999). Importantly, parasite-induced expression of A1 led to increased numbers of peritoneal macrophages and neutrophils, possibly by inhibiting apoptosis of these cells. Although it has not been directly addressed, increased expression of A1 may result from parasite-driven secretion of inflammatory cytokines such as GM-CSF (Orlofsky et al. 1999). Expression of heat shock protein (HSP) 65 in inflammatory macrophages after infection with *T. gondii* strains of low virulence also prevents apoptosis of these cells (Hisaeda et al. 1997). Furthermore, depletion of $\gamma\delta$ T cells abolished parasite-driven HSP65 expression and induced apoptosis in macrophages from *Toxoplasma*-infected mice (Hisaeda et al. 1997). This indicates that priming of $\gamma\delta$ T lymphocytes by *Toxoplasma* or its products under certain conditions regulates apoptosis of inflammatory macrophages in an indirect fashion. Whether the increase in HSP65 expression is mediated by secretion of inflammatory cytokines in vivo awaits further clarification.

3.2 Direct Inhibtion of Host Cell Apoptosis by *T. gondii*

In addition to indirect mechanisms, *T. gondii* has evolved strategies to directly inhibit host cell apoptosis (Nash et al. 1998; Goebel et al. 1999, 2001; Payne et al. 2003). Such inhibition requires the presence of intracellular parasites that may directly interfere with signaling cascades of the host cell (Fig. 1B). It is therefore restricted to parasite-positive host cells and is not observed in parasite-negative bystander cells (Goebel et al. 1999). Direct inhibition of host cell apoptosis has been predominantly investigated in vitro after treatment of host cells with proapoptotic stimuli to obtain high-level apoptosis. However, it has recently also been observed in peritoneal exudate macrophages from *Toxoplasma*-infected mice, indicating that it operates in vivo as well (Orlofsky et al. 2002).

Efforts have been undertaken to unravel the underlying mechanisms of this parasite-host cell interaction (Fig. 2). The results show that *Toxoplasma* interferes with activation of the caspase cascade, thereby abolishing cleavage of nuclear target proteins (Goebel et al. 2001; Payne et al. 2003). Although activation of caspase 9 and caspase 3 via the mitochondrial pathway was unequivocally inhibited by *T. gondii*, clear evidence is still lacking that the death receptor pathway involving caspase 8 activation is also targeted by the parasite. Inhibition of caspase 8 activity by *T. gondii* has been demonstrated in murine fibroblasts by Payne et al.

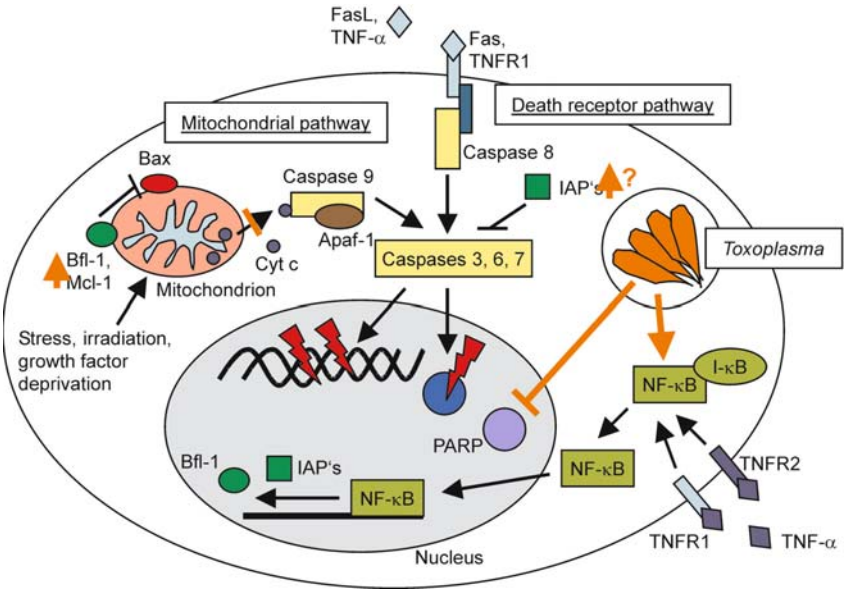

Fig. 2 Direct interference of intracellular *T. gondii* with signaling cascades regulating host cell apoptosis. Cell death may be initiated via the mitochondrial pathway or the death receptor pathway. Both pathways converge at the level of activated effector caspases, which then cleave cellular target proteins, leading to DNA fragmentation and apoptosis. A third major pathway initiated by the release of perforin and granzymes by cytotoxic NK cells and lymphocytes is not depicted. Intracellular parasites exert diverse mechanisms that are thought to contribute to direct inhibition of host cell apoptosis (indicated in *orange*): (i) Increased expression of antiapoptotic members of the Bcl-2 protein family, e.g. Bfl-1 or Mcl-1, (ii) inhibition of the cytochrome *c* release from mitochondria into the cytosol leading to decreased activation of the caspase 9 cascade, (iii) upregulation of IAPs possibly further inhibiting activity of caspases, (iv) activation of NF-κB by *T. gondii* in distinct cell types or under distinct conditions, thereby inducing the transcription of genes encoding antiapoptotic molecules, including Bfl-1 and IAPs, and (v) decreased cellular levels of PARP. The different antiapoptotic activities of *T. gondii* may be only partially related to each other

(2003) but was not found to occur in human histiocytic cells by others (Goebel et al. 2001), possibly indicating species- or cell type-specific differences. Furthermore, because the death receptor pathway partially overlaps with the mitochondrial pathway, at least in certain cell types (Kuwana et al. 1998; Scaffidi et al. 1999), inhibition of caspase 8 activity by *T. gondii* as described (Payne et al. 2003) does not necessarily exclude

the involvement of the mitochondrial pathway. The effect of *T. gondii* on the death receptor signaling pathway thus remains to be established.

Several mechanisms have been described that accompany decreased caspase activation in the presence of intracellular *T. gondii*. Inhibition of caspase 9 and caspase 3 activity clearly correlated with decreased cytochrome *c* release from mitochondria into the cytosol of *T. gondii*-infected human-derived tumor cells (Fig. 2; Goebel et al. 2001). Such interference may be at least partially mediated by increased levels of antiapoptotic proteins of the Bcl-2 family, because expression of Mcl-1 and Bfl-1/A1, but not other antiapoptotic Bcl-2 proteins, was increased after parasitic infection (Goebel et al. 2001; Molestina et al. 2003). Although this remains to be established, increased levels of these proteins may reduce the activity of proapoptotic Bax, Bak, and Bok to induce the cytochrome *c* release from mitochondria of infected cells (Adams and Cory 1998; Kaufmann and Hengartner 2001). In addition to increased levels of antiapoptotic Bcl-2 family members, inhibition of apoptosis by *T. gondii* may also be related to a parasite-driven increase of inhibitors of apoptosis (IAP) proteins, namely, NAIP1, IAP1, and IAP2 (Fig. 2; Blader et al. 2001; Molestina et al. 2003). Because IAPs are known to block apoptosis by direct inhibition of distinct caspases, such a mechanism would operate downstream of cytochrome *c* release and activation of caspase 8 (Deveraux et al. 1998). Degradation of the poly(ADP-ribose) polymerase (PARP) in the presence of intracellular parasites as described by Goebel et al. (2001) is also possibly involved in the inhibition of apoptosis (Fig. 2). PARP is well known as a target of activated caspases but also promotes cell death under certain conditions (Tanaka et al. 1995; Jacobson and Jacobson 1999). Although direct evidence is still lacking, it thus appears plausible that diminished PARP levels in *Toxoplasma*-infected cells may inhibit apoptosis in a caspase-independent fashion (Alano et al. 2004). In conclusion, at least three different mechanisms have been described that possibly abolish host cell apoptosis by intracellular *T. gondii*. Further analyses are clearly required to determine whether they represent redundant mechanisms that are of functional significance for inhibition of apoptosis in *T. gondii*-infected cells. It will also be of major interest to determine whether these different mechanisms are linked to each other or are independently regulated. In this context, it is noteworthy that blockade of apoptosis in murine fibroblasts by *T. gondii* required the activation of NF-κB (Payne et al. 2003). NF-κB regulates proinflammatory responses during microbial infection and also functions as a cellular prosurvival pathway (Wang et al. 1998; Van Antwerp et al. 1998). This is mediated by activating the transcription of genes en-

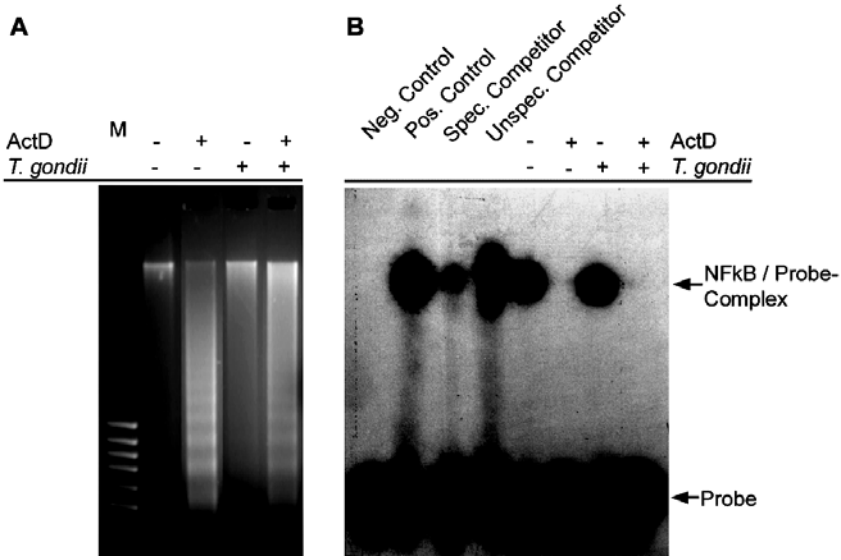

Fig. 3 Fragmentation of genomic DNA and DNA-binding activity of NF-κB in human-derived promyelocytic HL-60 cells after infection of *T. gondii*. HL-60 cells were infected with *T. gondii* or left uninfected as indicated and were then treated with actinomycin D (*ActD*) to induce apoptosis or left untreated. Eight hours after infection, genomic DNA was isolated and analyzed by agarose gel electrophoresis (**A**). In parallel, the binding activity of nuclear extracts to a radiolabeled NF-κB-specific oligonucleotide probe was assessed by electromobility shift assay (**B**). Note that ActD-induced DNA fragmentation was inhibited by *T. gondii* in the absence of DNA-binding activity of NF-κB

coding antiapoptotic molecules, including Bcl-2 and IAP proteins. Regulation of the antiapoptotic activity of *T. gondii* therefore appears to be partially linked. However, it should be noted that inhibition of apoptosis by *T. gondii* does not necessarily rely on activation of NF-κB. For example, in human-derived promyelocytic HL-60 cells, *T. gondii* inhibited actinomycin D-induced apoptosis in the absence of DNA-binding activity of NF-κB (Fig. 3). Furthermore, several groups have shown that *T. gondii* does not activate NF-κB and even inhibits activation of NF-κB induced after treatment of murine macrophages or fibroblasts with lipopolysaccharide (Butcher et al. 2001; Shapira et al. 2002). Therefore, NF-κB activation seems not to represent a general trait of intracellular *T. gondii*. Further studies are thus urgently required to unambiguously clarify the role of NF-κB in the inhibition of apoptosis by *T. gondii*.

Knowledge of the parasite molecule(s) that interfere with apoptosis-regulating signaling cascades of the host cell is still limited. Inhibtion of apoptosis required the presence of intracellular parasites, whereas intracellular replication of *T. gondii* was dispensable (Goebel et al. 1999). Notably, infection by a single viable parasite was thus sufficient to block apoptosis of the host cell. In addition, inhibition of apoptosis by *T. gondii* was reversible because it was abolished after killing the parasite (Nash et al. 1998). This indicates that the production and/or secretion of a *T. gondii* molecule are required to block apoptosis. *T. gondii* resides intracellularly in a specialized membrane-bound compartment, the so-called parasitophorous vacuole. It may therefore be hypothesized that the parasite molecule is either small enough to diffuse through pores within this membrane (Schwab et al. 1984) or is inserted into or even translocated across the membrane by yet unknown transport pathways (Cesbron-Delauw 1994; Beckers et al. 1994). Interestingly, Molestina et al. (2003) recently reported that the inhibitor of NF-κB activation, IκB, accumulates on the vacuolar membrane surrounding intracellular *T. gondii* in murine fibroblasts. This confirms the hypothesis that parasite molecules with access to the host cell cytoplasm may indeed interfere with signaling cascades of the host cell, thereby abolishing apoptosis.

3.3 Significance of Decreased Host Cell Apoptosis for Intracellular Survival

To grow and replicate, *T. gondii* relies on the sustained viability of its host cell. To date, however, the consequences of potential host cell apoptosis on the development of the parasite have not been directly addressed in detail. This question is not trivial because host cell apoptosis not only can disturb the intracellular development of the parasite but can also facilitate dissemination of certain pathogens (Gao and Kwaik 2000). Yamashita et al. (1998) reported that induction of apoptosis in *T. gondii*-infected target cells by CTL-mediated cytotoxicity does not lead to parasite death in vitro. In vivo, however, engulfment of apoptotic bodies containing viable parasites by phagocytic cells and subsequent elimination of the parasite may considerably contribute to parasite death due to apoptosis. Furthermore, whether such parasites are still able to invade new host cells also remains questionable. Because *T. gondii* actively invades its host cell and does not rely on the host cell's phagocytic machinery (Joiner and Dubremetz 1993), it appears unlikely that apoptosis significantly contributes to parasite dissemination within the host. It may thus be hypothesized that interference of *T. gondii* with apoptosis

of its host cell facilitates the intracellular development of the parasite and increases parasitemia (Fig. 1B). This view is supported by Orlofsky et al. (2002), who reported a decreased number of parasites in apoptotic macrophage populations. Furthermore, it has been shown that several viruses rely on the inhibition of host cell apoptosis to ensure the developmental cycle (Barry and McFadden 1998). However, further investigations are urgently required to assess whether such direct inhibition of host cell apoptosis is also required for the development of *T. gondii*.

As discussed above (see Sect. 3.1), apoptosis may also be reduced in uninfected bystander cells as a result of indirect mechanisms triggered after infection with *T. gondii* (Fig. 1C). Such reduced levels of apoptosis in distinct cell populations of the host may lead to an enhanced inflammatory response to the parasite (Hisaeda et al. 1997; Orlofsky et al. 2002). Inflammatory leukocytes limit parasite replication by T cell-independent effector mechanisms (Sher at al. 1993) but can also induce host mortality due to overwhelming immunopathology (Gazzinelli et al. 1996). Enhanced survival of inflammatory cells thus likely fulfills a dual role during toxoplasmosis depending on the host and parasite strain as well as the dose and route of infection. Indeed, depletion of $\gamma\delta$ T cells in mice infected with a low-virulence strain of *T. gondii* abolished parasite-triggered inhibition of apoptosis in peritoneal macrophages and reduced host survival (Hisaeda et al. 1997). This suggests that indirect inhibition of apoptosis after infection with *T. gondii* contributes to efficient parasite control. On the contrary, reduced cell death possibly also increases the inflammatory response to highly virulent *T. gondii* strains, thereby leading to increased immunopathology and host mortality (Orlofsky et al. 2002). From these experimental studies in mice, it is supposed that *T. gondii*-mediated inhibition of apoptosis by indirect mechanisms fulfills a crucial role in the regulation of the immune response and the outcome of infection.

4 Roles of Apoptosis During Toxoplasmosis

As discussed above, apoptosis may potentially fulfill a variety of innate and adaptive effector as well as regulator functions in the response to *T. gondii*. However, given the fact that parasite infection exerts a variety of different effects on apoptosis of its host-derived cells, what are the actual roles of apoptosis during toxoplasmosis?

4.1 Apoptosis as an Effector Mechanism Against *T. gondii*

After infection with *T. gondii*, $CD8^+$ and $CD4^+$ T lymphocytes with cytolytic activity against parasite-infected target cells have been isolated (Hakim et al. 1991; Montoya et al. 1996). Because $CD8^+$ T cells represent the more relevant effector cell type in the effective control of *T. gondii* (Suzuki and Remington 1988; Gazzinelli et al. 1992), cytotoxicity via the induction of apoptosis has been thought to represent an important effector mechanism to control parasite replication. However, this issue is obscured by the fact that both $CD4^+$ and $CD8^+$ T cells not only exert cytotoxic effects but also produce the protective cytokine IFN-γ (Suzuki et al. 1988). With perforin knockout mice, granule-mediated cytotoxicity of T lymphocytes and NK cells has indeed been shown to be dispensable to control parasite replication during the acute stage of infection (Denkers et al. 1997). In contrast, perforin-mediated target cell lysis partially restricted tissue cyst development within the brain and decreased susceptibility of mice during chronic *Toxoplasma* encephalitis (Denkers et al. 1997). This possibly indicates that cells harboring latent bradyzoite-containing tissue cysts are more susceptible to apoptosis than tachyzoite-containing host cells. It raises the interesting hypothesis that bradyzoites and tachyzoites differ in their ability to interfere with signaling cascades of the host cell, with only the latter considerably blocking apoptosis. Alternatively, distinct conditions within the brains of infected mice may also lead to the higher susceptibility of tissue cyst-containing host cells to CTL-mediated cytotoxicity.

Beside granule-mediated cytotoxicity, CTL may also induce apoptosis via the death receptor pathway (Tibbetts et al. 2003). Its impact on the control of *T. gondii* has not been thoroughly investigated. However, CTL-mediated apoptosis via this pathway represents an important regulator of the immune response rather than an effector function against intracellular pathogens (Lieberman 2003). It may therefore play only a minor role in parasite control during toxoplasmosis.

Apoptosis as a suicide program of the cell in response to intracellular infection with *T. gondii* seems not to play a significant role as an innate effector mechanism against the parasite. This may be largely due to the broad antiapoptotic effects of *T. gondii* (see Sect. 3.2). However, because the effect of the latent bradyzoite stage of *T. gondii* on host cell apoptosis is unknown, it cannot be excluded that apoptosis restricts parasite development during chronic toxoplasmosis.

In conclusion, apoptosis plays only a minor role in the innate and adaptive defense against acute *T. gondii* infection but may contribute to parasite control during chronic toxoplasmic encephalitis.

4.2 Apoptosis in the Pathogenesis of Toxoplasmosis

In contrast to a limited role in combating the parasite, apoptosis plays a crucial role in the pathogenesis of toxoplasmosis. Induction of high levels of apoptosis in splenocytes (Mordue et al. 2001; Gavrilescu and Denkers 2001), Peyer's patch T cells (Liesenfeld et al. 1997), and peritoneal macrophages (Hisaeda et al. 1997) after infection of mice with *T. gondii* may lead to defective immune responses to the parasite. Importantly, extensive apoptosis was associated with high-level parasitemia and increased susceptibility of mice to death after infection (Mordue et al. 2001; Gavrilescu and Denkers 2001; Liesenfeld et al. 1996; Hisaeda et al. 1997). Splenocyte apoptosis was clearly less evident after infection of mice with *T. gondii* strains of lower virulence leading to reduced parasite burdens (Lee et al. 1999). The level of apoptosis in T lymphocytes and possibly other leukocytes thus appears to correlate with the induction of pathology during toxoplasmosis. Such apoptosis may dysregulate the immune response, thereby leading to unrestricted parasitemia and damage of host tissues at least under certain conditions. In contrast, low or intermediate levels of T cell death may contribute to the parasite's ability to establish persistent infections. Thus a tight regulation of T cell death may have a critical impact on a stable parasite-host interaction during toxoplasmosis. This view is supported by the finding that apoptosis is able to restrict the intraocular inflammation in response to *T. gondii* (Hu et al. 1999). Apoptosis of T cells may thus not only restrict the parasite-specific immune response but also immunopathological changes of host tissue at least in mice. It will be of major interest to determine whether apoptosis fulfills similar roles in pathogenesis of human toxoplasmosis.

5 Concluding Remarks

During recent years, considerable progress has been made in our knowledge of the interaction of *T. gondii* with host cell apoptosis. It emerges that apoptosis triggered by *T. gondii* after infection of mice represents a

crucial factor in the pathogenesis of disease. For obvious reasons, much less is known on the role of apoptosis during human toxoplasmosis. Because the course of infection differs between mice and humans, it will be of major importance to unravel the impact of parasite-triggered apoptosis during human toxoplasmosis. Inhibition of apoptosis by *T. gondii*, on the other hand, may also represent a crucial factor for the parasite-host interaction and the course of disease. Although underlying mechanisms have been described, the exact cell biological and molecular bases and their regulation awaits further clarification. Particularly, the parasite molecules that interact with the apoptosis signaling cascades need to be characterized. This may then allow straightforward elucidation of the impact of decreased apoptosis on the course of disease. Unraveling the fascinating dual activity of *T. gondii* in host cell apoptosis and its regulation will undoubtedly further our understanding on the interaction of one of the most successful intracellular parasites.

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