6 SUMMARY

The obligate intracellular protozoan parasite *Toxoplasma gondii* causes persistent infections in a broad range of warmblooded vertebrate hosts, including up to 30% of the human population worldwide. Within its host, the parasite actively infects any nucleated cell, in which it may survive and replicate. The integrity of the host cell may be important for the intracellular survival of *T. gondii*. We therefore investigated the influence of *T. gondii* on the programmed cell death (apoptosis) of its host cells.

After infection of human-derived HL-60 and U937 cells with *T. gondii*, the rate of apoptosis was not elevated compared to uninfected controls. In contrast, after treatment of HL-60 cells with actinomycin D (actD) and U937 cells with TNF-α and cycloheximide, uninfected cells showed a clear induction of apoptosis. However, this *in vitro*-induced apoptosis was considerably inhibited after concomitant infection with *T. gondii*. Analyses on the single cell level by immunofluorescence microscopy revealed that parasite-positive cells from an infected HL-60 culture were significantly protected from programmed cell death.

To investigate the physiological prerequisites for the inhibition of host cell apoptosis, HL-60 cells were cultivated with either untreated, UV-irradiated or heat-inactivated parasites or were incubated with antigenic extracts from *T. gondii*. UV-irradiated parasites, which were able to invade the host cell but which were not able to replicate intracellularly, did suffice to promote the anti-apoptotic effect of *T. gondii*. In contrast, dead parasites or antigenic lysates from *T. gondii* did not significantly influence apoptosis of HL-60 cells. These results indicate that invasion of the host cell but not replication is necessary for inhibition of host cell apoptosis by *T. gondii*.

We further investigated the cellular mechanisms of the *T. gondii*-mediated inhibiton of host cell apoptosis. Neither the activation of the anti-apoptotic nuclear factor κB (NFκB) nor the expression of the heat shock protein (HSP) 60/65, which has been correlated with the inhibition of apoptosis in murine macrophages, was altered after parasitic infection. In contrast, it could be shown that *T. gondii* interferes with components of the main apoptosis pathways.

Proteins of the Bcl-2 family play an important role in regulating apoptosis-induction via the mitochondrial pathway. After infection of HL-60 cells with *T. gondii* and subsequent induction of apoptosis, however, expression of the antiapoptotic Bcl-2 was not affected. In contrast, expression of Mcl-1, another anti-apoptotic protein of the Bcl-2 family, was markedly increased after infection of HL-60 and U937 cells with *T. gondii* and following induction of apoptosis, as compared to uninfected controls. In addition, after treatment of HL-60 cells with LY 294002, an inhibitor of Mcl-1, the rate of actD-induced apoptosis increased in parasite-positive cells. Although the rate of apoptosis increased likewise in uninfected cells, these results suggest that Mcl-1 may be involved in the parasite-induced inhibition of apoptosis.

Since cytochrom c can be inhibited by Mcl-1 and activation of caspase 9 is mediated by release of cytochrome c from the mitochondria into the cytosol, we next investigated the subcellular distribution of cytochrome c in HL-60 cells after infection with *T. gondii*. Treatment of HL-60 cells with actD indeed induced release of cytochrome c from the mitochondria into the cytosol. However, after concomitant infection with *T. gondii* this translocation was clearly diminished. Analyses of Caspase 9 revealed that after induction of apoptosis in HL-60 and U937 cells the inactive procaspase 9 was more prominently detected in *T. gondii*-infected cells than in uninfected controls. This indicates that activation of caspase 9 was diminished after infection. Activation of the central caspase 3 that is activated by caspase 9 was likewise diminished after infection and subsequent induction of apoptosis as compared to uninfected cultures.

Interestingly, expression of the poly-(ADP-ribose)polymerase (PARP), a nuclear target protein of caspase 3-mediated cleavage during apoptosis and frequently used as a marker of apoptosis, was down-regulated after infection of HL-60 and U937 cells with *T. gondii*. Such parasite-induced down-regulation was similarly observed in cells that have been treated to undergo apoptosis and in untreated HL-60 and U937 cells. To our knowledge this represents the first description of a decreased expression of host cell PARP after infection with an intracellular pathogen, thus representing a novel example of the complex host-parasite interaction. Furthermore, after induction of apoptosis in HL-60 and U937 cells, proteolytic cleavage of the small amounts of PARP that were still detectable after parasitic infection was diminished as compared

to uninfected controls. This indicates that interference of *T. gondii* with activation of the caspase cascade during apoptosis indeed led to a reduced proteolysis of nuclear target proteins. This was further confirmed by the fact that the novel protein kinase C δ (nPKCδ), another target protein of caspase 3-mediated proteolysis was strongly cleaved in uninfected HL-60 cells after treatment with actD, while proteolysis was less prominent in cells concomitantly infected with *T. gondii*.

In conclusion, *T. gondii* interferes with at least two components of the apoptotic pathway: Mcl-1 and PARP. While modulation of Mcl-1-expression by *T. gondii* may represent the molecular basis for parasitic interference with cytochrome c-release from the mitochondria, activation of Caspase 9, 3 as well as cleavage of nuclear target proteins like PARP and nPKCδ, down-regulation of the expression of PARP represents an additional mechanism which may diminish *in vitro*-induced apoptosis. Further investigations are necessary to reveal the functional relevance of these anti-apoptotic mechanisms by *T. gondii*. The parasite only replicates within its host cell and outside its host cell is quickly opsonizated by antibodies and then phagozytosed and killed. Therefore, the inhibition of host cell apoptosis could contribute to the intracellular survival and persistence of the parasite.