Review

Host immune system against *Toxoplasma* infection

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Toxoplasma gondii is an intracellular protozoan parasite belonging to the subclass Coccidia. Toxoplasmosis is widespread in human beings and many other warm-blooded animals. The cats and other felines are the definitive hosts known to harbor the sexual stage of this parasite. Although *Toxoplasma* is an umcommon cause of disease in individuals with a normal immune system, immunocompromised hosts such as AIDS patients are at high risk of developing severe toxoplasmosis, especially toxoplasmic encephalitis as opportunistic infections (Sibley and Boothroyd, 1992).

Cell mediated immunity plays a crucial role in protective immune responses against *Toxoplasma* infection (Sethi et al., 1975; Nagasawa, 1984). However, the protective mechanisms involved in resisting infection with a strain of low virulence (Beverley strain) of *T. gondii* differ greatly from those involved in resisting infections with a highly virulent strain (RH strain) by the following results. When mice were immunized with homogenate of *Toxoplasma* before infection with a lethal dose of Beverley strain bradyzoites, the mice acquired resistance and survived. By contrast, vaccination with a sublethal dose of live Beverley strain bradyzoites was required for acquisition of resistance to infection with the highly virulent RH strain (Nagasawa et al., 1991). These findings seem consistent with observations that immunization with *Toxoplasma* homogenate along with adjuvant failed to prevent infection of mice by tachyzoites of the RH strain.

Heat shock protein

Exposure of cells to a variety of stressful conditions such as infection, immunization, elevated temperature, or stressful chemical intoxication lead to the transcription of a highly conserved set of genes and, subsequently, to the synthesis of a family of polypeptides called heat shock proteins (HSPs) (Lindquist, 1986; Schlesinger, 1986; Pelham, 1988). Among the various HSPs, a 65-kDa mycobacterial HSP has been identified as a target of T cells, including γδ T cells (van Eden et al., 1988; Res et al., 1988; Koga et al., 1989; O'Brien et al., 1989; Holoshitz et al., 1989; Haregewin et al., 1989). This HSP contains a highly conserved sequence and cross-reactivity with antigens from many of other microbes. T cells reactive to HSP65 derived from pathogen exhibit cytotoxicity to macrophages expressing host-derived HSP65 (Koga et al. 1989), suggesting that HSP contributes to the elimination of intracellular parasites as target antigens on the infected host cells. Moreover, HSP65 is known as one of the ligands of γδ T cells (O'Brien et al., 1991), which participate in protection against early phase of infections with intracellular pathogens (Hiromatsu et al., 1992; Raziuddin et al., 1992).

Relationship between the expression of HSP65 and protective immunity

When mice were immunized with *Toxoplasma* homogenate, HSP65 was detectable in peritoneal

macrophages from these mice after immunization but was not detectable from either unimmunized controls or *Toxoplasma* homogenate themselves. Furthermore, mice that acquired resistance against a high-virulence RH strain after the resolution of infection with Beverley strain bradyzoites strongly expressed HSP65 in their peritoneal macrophages. Moreover, a subset of γδ T cells has been shown to recognize HSP65 (O'Brien et al., 1989; Holoshitz et al., 1989; Haregewoin et al., 1989) and was found to increase rapidly in peripheral blood of patients with acute *T. gondii* infection (De Paoli et al. 1992; Scalise et al. 1992). Thus, it is not surprising that γδ T cells recognizing HSP65 and involved in protective immunity in some kinds of infection including toxoplasmosis. This T cell subset is thought possibly to represent a first line of defense against infection and is probably demonstrable in normal individuals. Furthermore, these HSP65-reactive γδ T cells should have been primed previously by contact with many different microbes or by exposure to HSP generated in host cells under various stressful conditions. We showed earlier that treatment of BALB/c mice with anti-Thy1.2 mAb one day before immunization with *Toxoplasma* homogenate led to an almost complete loss of the expression of HSP65. To determine the subsets of T cells responsible for induction of this protein, mice were depleted of γδ T cells, αβ T cells, $CD4^+$ T cells or $CD8^+$ T cells by treating with corresponding mAbs before immunization. From these experiments, γδ T cells were shown to be essential for the expression of HSP65, although $CD4^+$ αβ T cells also contributed to some extent (Nagasawa et al., 1994).

Neospora caninum

Neospora caninum is an intracellular protozoan parasite closely related to *T. gondii* (Dubey et al., 1988). It is frequently diagnosed as the cause of bovine abortion of epidemic proportion worldwide (Dubey and Lindsay, 1996). Vertical transmission contributes significantly to the spread of *N. caninum*, with most congenital infections resulting in the birth of healthy calves (Bjorkman et al., 1996; Pare et al., 1996; Schares et al., 1998).

Fas and Fas ligand

Fas and FasL interaction is closely associated with immune privilege and probably provides a barrier to prevent pathogens from damaging tissues in privileged sites. Moreover, the expression of Fas was up-regulated by IFN-γ on human peripheral blood T cells in vitro (Oyaizu et al., 1994). Numerous microbial pathogens have been reported to either induce or prevent apoptosis of their mammalian host cells (Zychlinsky and ansonetti, 1997). It was reported that *T. gondii* infection caused apoptosis in Peyer's patch T cells (Liesenfeld et al., 1997) and eyes (Hu et al., 1999) of mice. This apoptosis was Fas-dependent and mediated by IFN-γ. However, several papers have shown that *T. gondii* infection seems to protect cells from death induced by a number of agents (Goebel et al., 1998; 1999; Nash et al., 1998). Therefore, several contrasting views regarding the modulation of apoptosis following *T. gondii* infection exist.

We have earlier shown that *N. caninum* causes apoptosis in IFN-γ-treated BALB/3T3 clone A31 fibroblasts in vitro (Nishikawa et al., 2001). In the light of the close relationship between *T. gondii* and *N. caninum*, and the opposing reports on the modulation of apoptosis following *T. gondii* infection, in the present study we sought to investigate the mechanism(s) of the prevention/induction of apoptosis in IFN-γ-induced host cells infected with *T. gondii,* and *N. caninum* parasites in an in vitro system.

Apoptosis in host cells infected with *T. gondii* or *N. caninum*

The data we have generated from viability assay, FCM and TUNEL analyses, and DNA ladder observation clearly indicated substantial apoptosis

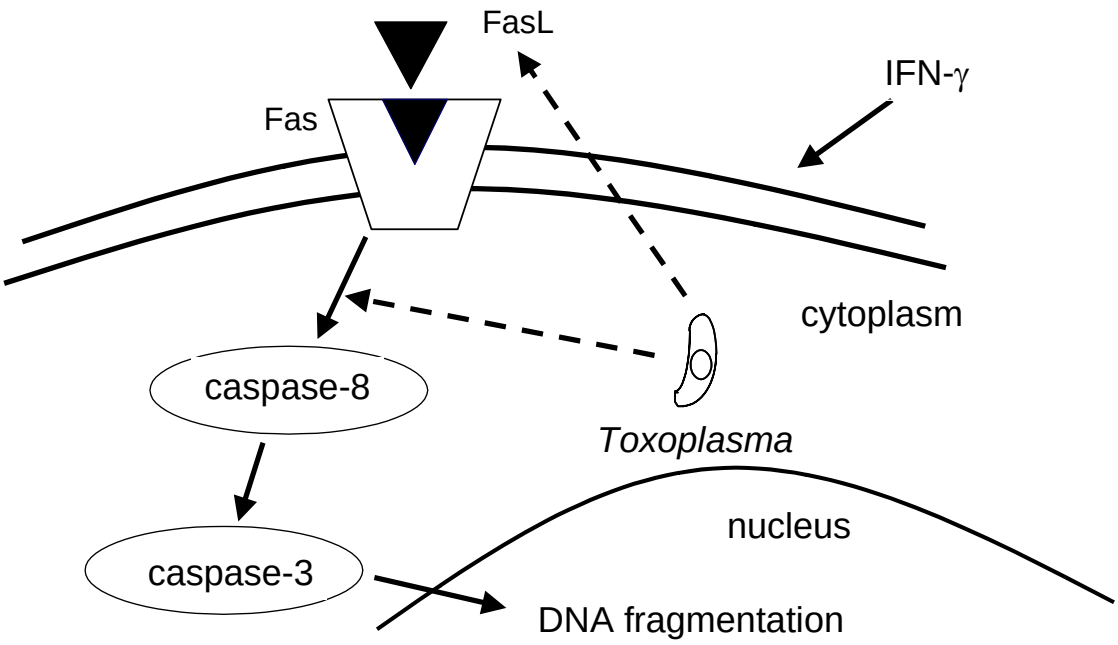

Fig. 1. Apoptosis signals in *Toxoplasma*-infected cells.

in IFN-γ treated and *N. caninum*-infected cells compared with *T. gondi*-infected cells. Killed *Neospora* parasites failed to induce apoptosis, implying that parasite invasion of host cells is essential in the induction of apoptosis by IFN-γ.

The significant activation of caspase-3 and -8, and the inhibition of apoptosis in the presence of caspase-3 and –8 inhibitors in *N. caninum*-infected and IFN-γ treated cells compared to *T. gondii*-infected cells, and the untreated and uninfected control group, suggest the crucial role of these proteins in the induction of apoptosis in *Neospora*-parasitized cells. In the light of earlier reports of mitochondrial releases of caspase-9 during apoptotic process (Marsh et al., 1995; Krajewski et al., 1999; Susin et al., 1999), it is highly likely that both caspase-9 and Bcl-2 play a supportive role in the induction of apoptosis in host cells infected with *N. caninum*. On the other hand, the resistance of *T. gondii*-infected cells to apoptosis may be attributed to prevention of caspase-3 and –8 activation (Fig. 1).

Similar levels of Fas-expression in cells infected with either *T. gondii* or *N. caninum*, and the post-administration of anti-Fas mAb revealed the induction of apoptosis in cells infected with either species, showing insignificant differences in infection rate in apoptotic cells. The Fas-mediated apoptosis in *T. gondii*-infected cells corroborates earlier findings of an IFN-γ induced Fas-dependent apoptosis in T cells in the Payer's patches in C57BL/6 mice following peroral infection with *T. gondii* (Liesenfeld et al., 1997). We have noted as well significantly higher FasL expression in *N. caninum*-infected cells compared to *T. gondii*-infected cells, and the decrease in cell viability in the presence of anti-FasL mAb was dose-dependent.

In the presence of IFN-γ, the number of *T. gondii* tachyzoites increased in host cells, but not in

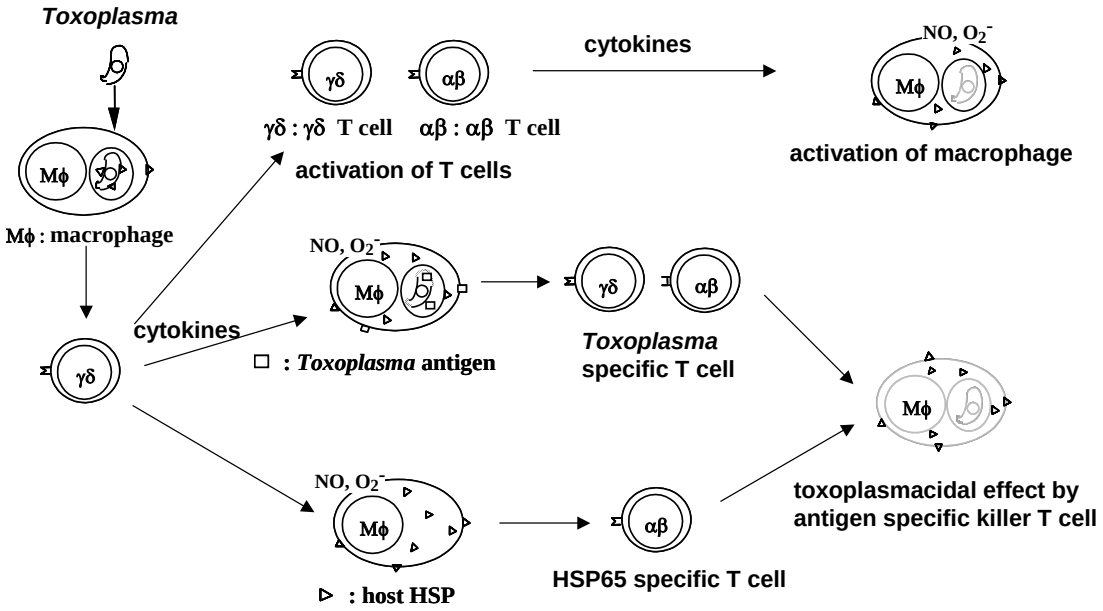

Fig. 2. Role of HSP65 for protective immunity against infection with *Toxoplasma gondii*.

N. caninum. It may be inferred then that the inhibition of apoptosis seems to prolong the life of the infected-host cells and promote the multiplication of *T. gondii* in the host. Also, the inhibition of apoptosis in *T. gondii*-infected cells may be interpreted to suggest increased susceptibility to the parasite.

Conclusion

Characterization of effective and regulatory functions of HSPs and apoptosis in other hosts and microbial systems should provide insight into mechanisms of virulence and protective adaptations that control virulence. Thus, it seems likely that HSPs and apoptosis could assume a critical importance in numerous host-parasite relationships, including resistance of host to otherwise destructively virulent parasites.

From these bases, the biological role of HSP65 and apoptosis may be as follows. As first step after infection with a low-virulence of *Toxoplasma*, circulating γδ T cells recognize either *Toxoplasma*-derived HSP65 or host-derived HSP65, and then they accumulate and activated. At the second step, macrophages activated by γδ T cells probably via certain cytokine pathways exhibit enhanced respiratory burst releasing noxious molecules, e.g. oxygen metabolites, a major protective mechanism against intracellular pathogens like *T. gondii*. As the third step, activated macrophages synthesize endogenous HSP65 for protection against these toxic molecules, for repairment of damaged functions of themselves or for effective antigen-presentation. Finaly, either γδ T cells and αβ T cells reactive to HSP65 or αβ T cells specific for *Toxoplasma* antigen further accumulate and are activated. Such T cells directly destroy the host macrophages or activate macrophages to kill the intracellular *Toxoplasma* parasites (Fig. 2). This hypothesis is considered by the role of host cell-derived HSP. However, it has been reported that highly virulent strain of *Toxoplasma* expressed high level of HSP compared with low virulent strain. Because a function of HSP is to rescue the cell

death, HSP derived from highly virulent strain of *Toxoplasma* has a function to suppress apoptosis which are effective to eliminate intracellular parasites in host cells. Moreover, it is of interest to investigate the relationship between HSP and apoptosis in protective immunity against *Toxoplasma* infection.

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