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Molecular Modifications of Host Cells by *Toxoplasma gondii*

HENRYKA DŁUGOŃSKA

Department of Immunoparasitology, Institute of Microbiology and Immunology,
University of Łódź, ul. Banacha 12/16, 90-237 Łódź, Poland,
e-mail: hdlugo@biol.uni.lodz.pl

A b s t r a c t

Toxoplasma gondii, the etiological agent of toxoplasmosis, is an *Apicomplexa* obligate intracellular protozoan parasite, which is able to infect any nucleated cell of numerous endothermic vertebrates. The combined abilities to actively penetrate host cells and perfectly control the fate of the parasite-containing vacuole (parasitophorus vacuole, PV) contribute to the remarkable global success of *Toxoplasma* as an intracellular parasite. Very broad host range and the relative ease of growth both in cell cultures *in vitro* and *in vivo* suggest that the parasite is able to manipulate the host cell apoptotic machinery. The article describes different aspects of host-parasite interplay focusing on molecular modifications of infected host cells.

K e y w o r d s: *Toxoplasma gondii*, host-parasite interplay, host cells modifications

Host cells as microontohabitat for *Toxoplasma gondii* – life in parasitophorus vacuole

Toxoplasma gondii invades both phagocytic and nonphagocytic cells by an active multistep and carefully orchestrated process including attachment, penetration and internalization.

During invasion, the host cell is essentially passive and no change is detected in membrane ruffling, the actin cytoskeleton or phosphorylation of host cell proteins (Dobrowolski and Sibley, 1997).

Table I
Phagocytosis and invasion (penetration) of host cells by *T. gondii*
(according to Dobrowolski and Sibley, 1997)

Process	Phagocytosis	Invasion
Duration	2–5 min	15–40 sec
Host cells:		
type	macrophages, granulocytes	all nucleated cells
actin condensation	yes	no
membrane ruffling	yes	no
tyrosine phosphorylation	yes	no

Toxoplasma exhibits several highly characteristic forms of motility: circular gliding, twirling and helical gliding and only the latter leads to cell invasion, the parasite gets screwed into host cell like cork-screw (Sibley, 2003). The invasion is initiated by contact between the apex of *T. gondii* and the host cell surface, involving both many host receptors (proteoglycans: heparin and heparan sulphate, β-integrins) and parasite ligands: surface antigens (SAG), surface antigen-related sequences (SRS), microneme proteins (MIC) and laminin (Furtado *et al.*, 1992; Jacquet *et al*., 2001; Manger *et al.*, 1998; Ortega-Baria and Boothroyd, 1999). While *T. gondii* uses sulphated glycosaminoglycans such as heparin and heparan sulphate as target molecules for binding to their host cells, tightly related apicomplexan parasite *N. caninum* prefentially interacts with chondroitin suphate residues (Naguleswaran *et al*., 2002).

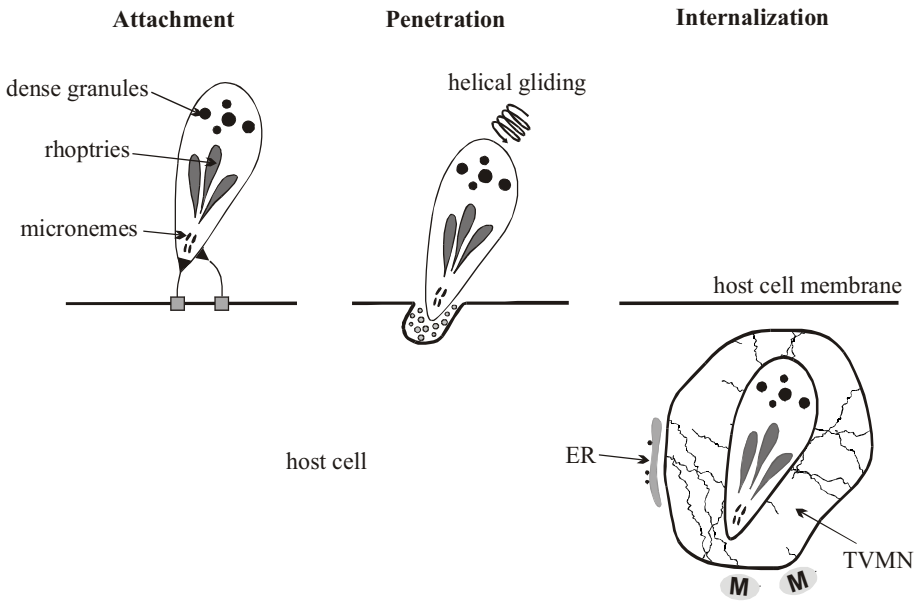

Fig. 1. The invasion of host cell by *Toxoplasma gondii*.
Parasite's (▼) and host's (■) cell components involved in attachment, PVM – parasitophorus vacuole membrane, M – mitochondria, ER – endoplasmic reticulum with ribosomes, TVMN – tubulovesicular membranous network (modified from Bonhomme *et al*., 1999)

During invasion, the contents of three separate secretory organelles of *T. gondii* are discharged sequentially (Carruthers and Sibley, 1997; Dubremetz, 1998), Fig. 1. First, small apical vesicles called micronemes release a family of adhesive proteins, involved in recognition of and attachment to the host cells. The second wave of exocytosis occurs from rhoptries whose content is responsible for forming (biogenesis) of nascent parasitophorus vacuole (PV). Rhoptry proteins are commonly located on the host cytoplasmic side of PVM (parasitophorus vacuole membrane), suggesting their role in host-parasite biochemical communications. The cytoplasmic face of PVM is lined with continuous layer of host mitochondria (M) and endoplasmic reticulum (ER). The latter has no ribosomes on the side oriented to PVM. *T. gondii* tachyzoites have evolved a surface-associated proteolytic machinery which extensively modifies their secretory proteins (microneme and rhoptry origin), contributing to the adhesive properties of the parasite (Naguleswaran *et. al.*, 2003). Finally, dense granule proteins (GRA1-GRA8) are released to fully formed vacuole and the secretion is continued during *T. gondii* replication. They participate in modifying of the vacuole and can be dispatched to various targets: PVM, reticular network in PV, vacuolar space and even host membrane and cytosol (Bonhomme *et al.*, 1999). Consistent with the formation of a specialized compartment-parasitophorus vacuole, the invasion of *T. gondii* into hosts cell leads to exclusion of the transferrin receptors and rab5 as markers of early endosomes (Mordue and Sibley, 1997). Furthermore, within PVM have been not detected late endosomal markers: rab7 and cation-independent mannose 6-phosphate receptor (CI-M6PR), lysosomal marker LAMP1 (lysosome-associated membrane protein 1) and proton pump (Joiner *et al*., 1990; Mordue and Sibley, 1997; Mordue *et al.*, 1999). Entering of the PV by parasite is associated with the increase of K^+ level and $sPLA_2$ II (phospholipase 2) activity in host cytosol and the host membrane becomes hyperpolarized (Bonhomme *et al.*, 1999).

Following entry*, Toxoplasma* rapidly divides by binary fission, forming sequential pairs of daughter cells (endodyogeny). After six to eight parasite divisions the host cell lyses and released tachyzoites infect surrounding cells and tissues. Chronic infection is associated with differentiation into bradyzoites that form cysts preferentially in central nervous system, skeletal muscle and eye. At the beginning of cyst formation, the PVM remains relatively smooth, making a few long tubular invaginations inside the cyst. Irrespective of their size and age, the tissue cysts are present within intact brain cells that provide continuing niche for parasite's survival (Beyer *et al*., 2002).

The infection with *Toxoplasma gondii* occurs usually by the accidental ingestion of cysts present in contaminated meat or oocysts excreted by cats. Chemokines released by intestinal epithelial cells recruit polymorphonuclear neutrophils, dendritic cells, macrophages and lymphocytes. The relation and influence of the parasite on diverse host cells will be presented.

Toxoplasma gondii and various host cells

Neutrophils. Several lines of evidence suggest that neutrophils are essential for survival during the first few days of infection, but their parasiticidal effect is not associated with reactive oxygen intermediates. Neutrophils were recently identified as a source of several proinflammatory cytokines: IL-12 (interleukin 12), TNF-α, (tumor necrosis factor α) and chemokines: MIP-1α, MIP-1β (macrophage inflammatory protein 1α, 1β) that are stored in preformed pools in secretory or possibly gelatinase granules. Recruited rapidly (2–3 h after infection) and in large numbers to the site of infection, neutrophils are ready to release cytokines and chemokines that are very important regulators of cell recruitment and activation, among them immature dendritic cells (Denkers *et al.*, 2004, Kasper *et al.*, 2004).

Dendritic cells (DC). DC, previously thought to be only very efficient antigen-presenting cells, have been transformed recently into architects of immunity. Many studies have implicated that the interaction parasite-dendritic cell is a key element of the host response, because dendritic cells play a crucial role not only in inducing cellular immunity, but also in directing its profile – Th1 or Th2 (T helper 1 or 2 lymphocytes). The ability of DC to produce IL-12 relies on synergy of MyD88 (a Toll-like receptor adaptor molecule) and CCR5 (CC chemokine receptor 5) signaling (Aliberti and Sher, 2002; Denkers *et al.*, 2004). Immature DC in response to CCR1 and CCR5 ligands, migrate to inflammatory sites, where they process the antigen, mature, down-regulate CCR1 and CCR5 and up-regulate CCR7, which facilitates them homing into T-cell areas of secondary lymphoid organs. It is worth noticing that, almost as a rule, invaded by toxoplasms DC either fail to respond or are functionally suppressed. Nevertheless, DC can be activated by tachyzoite extracts, secreted parasite components or through cross-priming by other infected cells (Sher *et al.*, 2003). Aliberti and Sher (2002) have proposed two general mechanisms by which *T. gondii* soluble extract (STAg) could trigger IL-12 production. The first hypothesis (autocrine stimulation model) argues both the direct activation of IL-12p40 gene expression and CCR5 ligands production. The chemokines produced interact with CCR5, amplifying IL-12 production. In the alternative hypothesis (chemokine mimicry model) the second enhancing signal for IL-12 production is provided by parasite component, CCR5 ligand mimic. The authors emphasize that G-protein coupled CCR5 signaling is an enhancing factor to the weak signal from undetermined parasite triggering molecule. The most fascinating question is, why CCR5-dependent G-protein signaling is stimulatory in the case of *T. gondii*, but inhibitory in the case of other microbial stimuli. After exposure to STAg, DC are unresponsive 5–7 days to further stimulation. The mechanisms of the paralysis are based on lipoxin A4, an eicosanoid, arachidonate inhibitor of acute inflammation produced by macrophages under *T. gondii* stimulation. Lipoxin, by binding to formyl-peptide receptor ligand -1 (FPRL-1), down-regulates CCR5 expression on DC and consequently, IL-12 production. This phenomenon, termed "DC paralysis", might be unique for *Toxoplasma*, because lipoxin had no inhibitory effect on IL-12 production triggered by other microbial products, for instance LPS. DC paralysis is a likely mechanism to avoid proinflammatory cytokine production (Scott and Hunter, 2002). Interestingly, human monocyte-derived DC discriminate between alive and killed tachyzoites. Only the first ones up-regulate CD28 and CD40 on DC and initiate IL-12 synthesis, whereas a *Toxoplasma* lysate was a poor inducer of IL-12 (Subauste and Wessendarp, 2000). Fischer *et al.* (2000) found that *Toxoplasma gondii* triggered expansion and maturation of DC from their progenitors in central nervous system and emerged as a major source of IL-12, suggesting a role of long-term IL-12 production in protection. The long-lasting presence of activated DC in infected brain might contribute to the chronicity of the intracerebral cellular response in *Toxoplasma* encephalitis. The traditional concept of the brain as "immunoprivileged" organ should be modified.

Macrophages. Macrophages, evolutionary ancient cells, play a key role in defense against infection. Tachyzoites of different *T. gondii* strains, but not bradyzoites, induce chemokine MCP-1 (macrophage chemotactic protein -1) that recruit intensively macrophages during acute infection (Brenier-Pinchart, 2002). Despite the fact that macrophages are potent effectors of innate immunity (phagocytosis, killing by reactive oxygen and nitric intermediates, limiting iron and tryptophane resources, producing proinflammatory cytokines) the cells serve as hosts for *T. gondii*. After macrophage penetration, the parasite inhibits acidification of the parasitophorus vacuole, actively suppresses proinflammatory cytokine synthesis by interference with intracellular signaling pathway or stimulates production of counter-regulatory cytokines. When macrophages are infected with the parasite, the cells fail to produce TNF-α and IL-12 production occurs only later, after delay of approximately 24 h. *Toxoplasma* infection induces rapid activation of transcription factors such as STAT1 and NFκB, but blocks their translocation from cytoplasm to nucleus for 24 hours (Butcher *et al.*, 2001). After nuclear import blockade, macrophages begin to release IL-12, but remain actively suppressed in their ability to produce TNF-α. *T. gondii* triggers two independent cell pathways leading to IL-12 production: the first

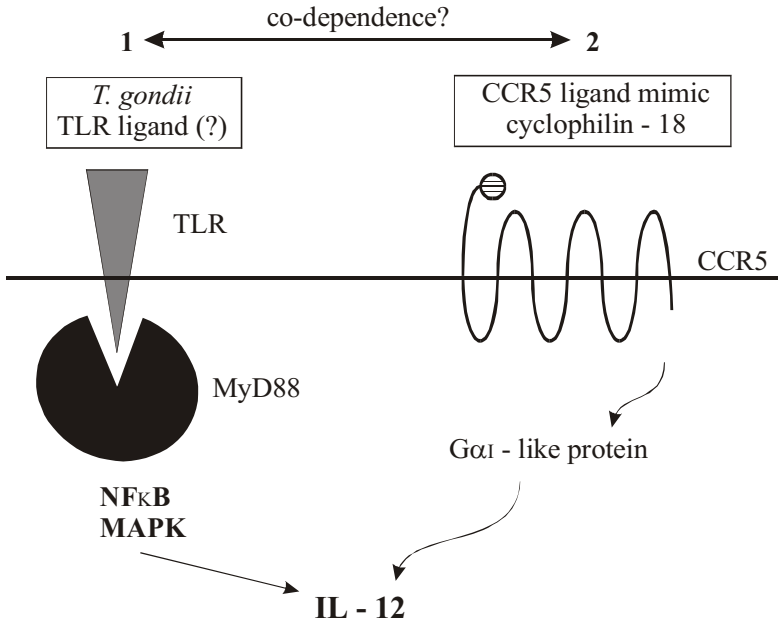

Fig. 2. Production of IL-12 by *Toxoplasma gondii* – triggered host immune cells: MyD88- and CCR5 – dependent pathways.
TLR – Toll like receptor, MyD88 – transducer molecule, NFκB – nuclear factor kappa B, MAPK – mitogen-activated protein kinase, CCR5 – CC chemokine receptor 5, IL-12 – interleukin 12 (modified from Denkers, 2003; Denkers *et al*., 2003)

(in macrophages, dendritic cells and neutrophils) through TLR receptor and transducer molecule MyD88 and the second – in dendritic and possibly other cells through CCR5 and G-protein (Denkers *et al*., 2003).

The parasite is known as a strong activator of innate and acquired immunity, associated with high levels of IL-12, IFN-γ and TNF-α, all important in protective immunity. The view that the parasite is solely a proinflammatory pathogen is oversimplistic. Many observations clearly indicate active suppression mediated by *Toxoplasma.*

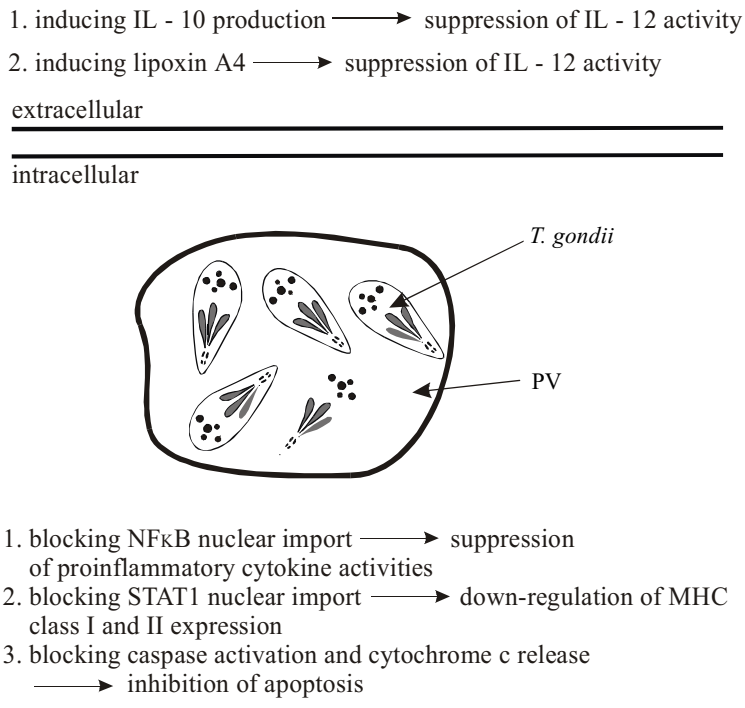

Fig. 3. Immunosuppression in toxoplasmosis – extracellular and intracellular pathways.
IL-10 – interleukin 10, IL-12 – interleukin 12, NFκB – nuclear factor kappa B, STAT1 – signal transducer and activator of transcription 1, MHC – major histocompatibility complex, PV – parasitophorus vacuole (modified from Denkers, 2003)

The ability to delay or dampen the proinflammatory cytokine synthesis allows *T. gondii* for early expansion and prevents the host from immunopathology due to overproduction of those cytokines. By comparing the immune response of mice infected with different *Toxoplasma* strains, it was observed that highly virulent strain RH elicited IFN-γ hyperproduction, extensive apoptosis and marginal $NO^{\bullet}$ production, whereas the infection by low-virulent strain ME49 induced low levels of IFN-γ, less apoptosis, but higher levels of $NO^{\bullet}$. The ingestion of IFN-γ-induced apoptotic cells triggers an alternatively activated macrophages population, that owing to their minimal $NO^{\bullet}$ production could support the intracellular growth of the virulent parasites (No *et al.*, 2004). As shown by Lüder *et al.* (2003a) the infection *in vitro* of primary bone marrow-derived macrophages or monocyte/macrophage line RAW264.7 with mouse low-virulent strain down-regulated of IFN-γ or LPS-induced $NO^{\bullet}$ synthesis as well.

The infection of human monocytes (and other cells) with *T. gondii* first induces a hyperpolarisation of host plasma membrane, followed by transient polarization of PVM, which decreases during parasite replication. The observed depolarization might be controlled by H^+ and H^+/K^+ ATPases as was shown by use of proper ATPases inhibitors (Bouchot *et al.*, 2001).

Lymphocytes. Initiating and sustaining strong T lymphocytes – mediated immunity is crucial in preventing the emergence of *T. gondii* as a serious pathogen. Parasite-specific Th1 lymphocytes release high levels of IFN-γ, a pitoval protective cytokine in *Toxoplasma* infections, which is required to prevent cyst reactivation. Besides, both CD8+ and CD4+ lymphocytes show cytolytic activity against host cells infected with the parasite (Denkers and Gazinelli, 1998).

Mast cells. The studies performed on experimental model, a wild mouse-like autochthonous rodent from South America *Calomys callosus*, extremely susceptible to toxoplasmosis, demonstrated that mast cells were involved in acute phase of inflammatory response. After 1 h of infection, a significant influx of mastocytes into peritoneal cavity was observed. Their morphology suggested degranulation process, triggered probably by secreted parasite antigens. Degranulation initiated a remarkable increase in neutrophils influx after 12 h post-infection (Ferreira *et al.*, 2004)

Apoptosis as a strategy of modulating *T. gondii* – host interactions

Toxoplasma is able to induce and inhibit apoptosis using different strategies. The factors determining the direction of the opposite effects have not been determined, but might be dependent on the virulence of the parasite, host cell type and stage of its infection.

Programmed cell death (*i.e.* apoptosis) is a complex and highly regulated process of multicellular organisms. Besides its critical roles in development, aging and homeostasis, apoptosis is an important defense mechanism against viruses, bacteria and parasites. Parasitic pathogens have evolved diverse strategies to interfere with cell host apoptosis and thereby modulating the host's immune response, facilitating intracellular survival and promoting dissemination within the host (Lüder *et. al*., 2001b).

There are two primary pathways of apoptosis, induced by extrinsic (death receptor activation) or intrinsic (DNA damage) stimuli. The first one triggers the caspase 8 dependent pathway, while the second activates caspase 9 pathway (mitochondrial pathway) following the release of cytochrom c into cytoplasm that promotes the formation of the apoptosome, a complex composed of cytoplasmatic protein APAF1, cytochrome c and caspase 9. Activated by proteolytic processing (trans-cleavage) within apoptosome caspase 9 acts on procaspase 3 and its activation initiates the executioner phase of apoptosis resulting in cleavage of many nuclear, plasma membrane and cytoskeletal targets (Sinai *et al*., 2004). The mitochondrial pathway of apoptosis is particularly interesting with regard to *T. gondii,* because of intimate association of parasitophorous vacuole with host mitochondria (Sinai and Joiner 2001, Goebel *et al*., 2001). Why does *T. gondii* block the apoptosis? To survive long time in its host. *T. gondii* is auxotrophic to critical metabolites, including purines, certain amino acids (Fox *et al.*, 2004) and cholesterol (Coppens *et al.*, 2000), which have to be supplied by the host cell. By blocking apoptosis, the rapidly growing parasite ensures the steady providing of metabolites for its replication. Inhibition of apoptosis seems to be also important for stage conversion from aggressive tachyzoites with high growth rate to dormant, slowly multiplying bradyzoites and establishment of chronic phase of infection (Tenter *et al*., 2000). Keeping the infected host cell alive is likely to be critical and this protection from apoptosis is implied from the observations *in vivo* that tissue cysts containing bradyzoites may survive for years or even decades. Manipulation of apoptosis could reveal in the immune response, both natural and adaptive. Apoptotic cells, sending the signal "eat me" (for example, by exposure of surface phosphatidylserine), are *in vivo* rapidly cleared by macrophages. Recent observations

of Fox *et al.* (2004) suggest that arginine depletion of infected host cell can trigger bradyzoite differentiation. These findings are consistent with a model of host-parasite evolution that favoured host control of bradyzoite induction over parasite control of tachyzoite proliferation, trading off virulence for increased transmission. Interestingly, *T. gondii* also induces apoptosis (of T lymphocytes, macrophages), possibly initiated by Fas-FasL interaction. The results of Liesenfeld *et al*. (1997) indicate that peroral infection with *T. gondii* IFN-γ leads to Fas dependent apoptosis of CD4+ and CD8+ lymphocytes in Peyer's patches, induced by IFN-γ. The Fas-FasL interaction on lymphocytes and on ocular tissues is involved in pathogenesis of acquired ocular toxoplasmosis. Intraocular inoculation *of T. gondii* induced inflammation associated with increase in Fas and FasL expression (Hu *et al.*, 1999). The dual activity of *Toxoplasma* on host cell apoptosis might require an exclusively balanced interplay between pro- and anti-apoptotic signals of the infected host. *T. gondii* as an obligate intracellular parasite needs continuous supply of essential metabolites and host cell apoptosis limits parasites replication. Induction of apoptosis of certain immune cells down-regulates parasite-specific immune response resulting in increased parasites survival and prevention of immunopathological processes, so induction of apoptosis in the course of infection may be beneficial for the parasite and for the host (Lüder *et al*., 2001b).

Another aspects of *T. gondii* – host interplay

One of the evasion mechanisms induced by *T. gondii* in host cells is the downregulation of MHC (major histocompatibility complex) class II expression. Protective immunity against *T. gondii* is mediated by specific CD8+ and CD4+ lymphocytes. The first step of priming and activation of these cells is presentation of antigenic peptides in the context of MHC class I and class II molecules. According to a classical paradigm in immunology, antigens presented by class I molecules are classified as cytosolic (endogenous) and those presented by class II molecules as extracellular (exogenous) derived ones. *T. gondii*, like other intracellular pathogens, has evolved different strategies to interfere with class II-restricted presentation to evade host immune response and survive intracellularly. PV containing viable parasites does not fuse with lysosomes and does not acidify, thereby avoiding degradation by proteases. In addition to mediating formation of non-fusogenic PV, *T. gondii* down-regulates MHC class II expression on human melanoma cells (Yang *et al.*, 1996), primary mouse macrophages (Lüder *et al.,* 1998) and neural antigen-presenting cells (Lüder *et al*., 2003b). In contrast, the expression of MHC class I molecules was not influenced by the parasite (Lüder and Seeber, 2001). Despite the interference of *T. gondii* with MHC class II presentation pathway, infection of immunocompetent hosts induces priming and expansion of CD4+ lymphocytes. Most *T. gondii* proteins which elicit CD4+ T cell responses are secretory proteins, particularly dense granule proteins and the major surface antigen SAG1 (Fischer *et al.*, 1996; Reichmann *et al.*, 1997; Prigione *et al.*, 2000) which are recognized in the context of defined MHC class II molecules.

Lüder and Seeber (2001) proposed that secretory proteins as well as those derived from non-viable parasites or associated with cellular debris from lysed host cell are endocytosed by APC (antigen presenting cell) and then presented, to some extent, *via* the conventional MHC class II pathway. However, due to the fact of PV fusion incompetence and down-regulation of MHC class II molecules, host APC infected by live parasites are not recognized by CD4+ lymphocytes thus facilitating intracellular *T. gondii* survival. Since surface MHC class II molecules are excluded from the nascent PVM during invasion of the host cell, alternative loading of antigenic peptides within early endosomes seems unlikely. In both humans and mice, *T. gondii* infection provides a potential stimulation for the generation of CD8+ effectors capable of lysing infected target cell (Denkers and Gazinelli, 1998).The problem is, how *T. gondii* antigens reach the MHC class I pathway in infected cells. The PVM is believed to act as a molecular sieve, allowing bi-directional passive transport of molecules up to 1300 Da. This would mean that only small peptides degradated by parasite proteases or intact proteins actively transported could gain access to host cell MHC class I pathway. Other mechanisms can be also not excluded, *e.g.* direct loading of host cell MHC class I molecules by released parasite antigens at the time of invasion. Recently, an alternative, non-classical route of MHC class I antigen presentation, called "cross-presentation", has been widely accepted. Exogenous antigen can be processed extracellularly by serum or surface host cell proteases and load empty MHC class I molecules or alternatively can be internalized (endocytosis, macropinocytosis and phagocytosis), transported to intersection between MHC class I and II pathways, degradated by proteases and resulting peptides are loaded onto MHC class I molecules, in endoplasmic reticulum by TAP (transporter associated with antigen processing) or in endosomes, independent of TAP (Lüder and Seeber, 2001).

After infection the mammalian cells acquire a novel dynamic compartment, *i.e.* parasitophorus vacuole, which contains live and dividing parasites. The replication of *T. gondii* within parasitophorus vacuole must coincide with a significant increase in membrane mass (biogenesis of nascent parasites membranes and enlargement of PVM). Confocal microscopic analyses demonstrated that host lipids were compartmentalized into parasite endomembranes or concentrated in discrete lipid bodies, distinct from the parasite secretory organelles. (Charon and Sibley, 2002). The mechanism(s) by which host-derived lipids are transferred across the PVM to the parasite remains unknown. One of a few possibilities is carrier protein-mediated transport, for example by using host low-density lipoprotein receptors. The exposure of *T. gondii* – infected cells to low-density lipoproteins led to their internalization in host endosomes and lysosomes and after 20 min they were transported through PVM inside vacuolar space, then surrounded by PVM. The phenomenon illustrates a new feature of the parasite to have an access to essential nutrients in lysosomes without being exposed to digestive hydrolases (Lüder *et al.*, 2001a). *Toxoplasma* is also capable of lipid biosynthesis, at least fatty acid biosynthesis. Although cholesterol-enriched organelle – rhoptries discharge at the time of host cell entry and contribute to PWM formation, host but not rhoptry cholesterol is incorporated into the forming PVM, through a caveolae-independent mechanism. Depleting host cell membrane cholesterol inhibits parasite internalization by reducing rhoptry proteins release (Coppens and Joiner, 2003).

The analysis of transcriptional profile of human fibroblats infected with *Toxoplasma* or bacterial intracellular pathogens revealed two genes that were specifically up-regulated by toxoplasms: gene for transferrin receptors and gene for MacMARCKS (responsible for integration of Ca^{2+} – calmodulin and protein kinase-dependent signals) (Gail *et al*., 2001).

Although nearly one-third of the world population has been exposed to *Toxoplasma*, the infection occurs mainly in place of precarious sanitary conditions and nutrition. Using an experimental toxoplasmosis model the genotoxicity of the parasite *in vivo* was evaluated in isogenic mice under dietary restriction and submitted to a treatment with sulphonamides, which are widely used for treatment of the toxoplasmosis. The results indicated that dietary restriction induced DNA damage in peripheral blood cells of infected mice, whereas the liver and brain cells were not influenced. Sulphonamide therapy did not show any genotoxicity, as detected by the comet assay (Ribeiro *et al.*, 2004).

Does *Toxoplasma gondii* manipulate us?

The infection with *T. gondii* modulates not only the host immune responses in both acute and chronic phases of toxoplasmosis but also physiological and behavioural changes. In experimentally infected mice a decline in serum thyroxine (T4) was observed (Stahl and Kaneda, 1998) that is likely do to perturbation of the pulsative release of TSH from pituitary. Besides, concomitant immunization of the mice with non-related soluble antigens leads to predominant production of IgG2a specific antibodies, instead of IgG1 as seen in non-infected animals. Such a change could significantly influence the reactivity of hosts vaccinated with different microorganisms antigens (Nguyen *et al.*, 1998). Animals infected with *T. gondii* may show a variety of neurological and behavioral symptoms, including changes in activity, learning and memory. The transmission of parasites during early pregnancy causes mental retardation (Webster, 2001).

The high prevalence of *T. gondii* in the human population offers the opportunity of studying the influence of the parasite on human behaviour. Flegr *et al.* (1995) found a positive correlation between duration of latent toxoplasmosis and the intensity of superego (willingness to accept group moral standards) decrease. The studies, which have been done on individuals with schizophrenia, reported a higher percentage of seropositive in affected group and increased levels of cognitive impairment compared to age- and severity-matched individuals with schizophrenia but seronegative (Torrey and Yolken, 2003). In conclusion, latent toxoplasmosis, although frequently dismissed as asymptomatic and clinically unimportant in both humans and rodents does alter host behaviour, both in rodents, where altered responses may be proposed to benefit the parasite (enhancing transmission rate through food chain), and humans, where altered responses may arise as a side-effect with non current adaptive significance (Webster, 2001). Are we and other endothermic vertebrata puppets of *Toxoplasma?*

Concluding remarks

Stability of the host-parasite relationship during *T. gondii* infection demands that the parasite avoids elimination by effectors of host immunity but also demands that triggered immunity is enough to prevent

the host from uncontrolled parasite replication and death. The fact that one third or even more of the human population in the world is chronically and asymptomatically infected with *Toxoplasma* indicates that the parasite and its hosts have co-adapted very well to achieve a stable balance.

In addition to its clinical importance, *T. gondii* is recently increasingly becoming an attractive model organism not only for investigating apicomplexan parasites but also basic cellular functions of interest to broader scientific community beyond parasitologists, physicians and veterinarians (Kim and Weiss, 2004). Investigations of fundamental pathways in parasites may provide insight into organisms where direct experimentation is difficult or impossible. Besides, such studies might lead to the identification of crucial structures that can be then used to target parasites without damaging the host cells, for example pyridinylimidazoles block selectively parasite replication, probably by targeting an enzyme, p38 mitogen-activated protein kinase (MAPK) homologue in *Toxoplasma gondii*, but not MAPK in host cells (Wei *et al.*, 2002). *Toxoplasma gondii*, an ancient parasite, enjoys its second youth?

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