Unexplained Liver Damage, Cryptogenic Liver Cirrhosis, and Steatohepatitis May Be Caused by Latent Chronic Toxoplasmosis

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Conflict-of-interest statement: The authors declare that there is no conflict of interest regarding the publication of this paper.

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Received: March 26, 2019
Revised: April 2, 2019
Accepted: April 3, 2019
Published online: April 21, 2019

ABSTRACT

*T. gondii* is a globally distributed intracellular protozoan parasite affecting approximately 5-90% of human population and causing a variety of so far neglected diseases and clinical entities in both immunocompromised and immunocompetent individuals. Acute infection of the parasite in mice caused marked reduction in serum butyrylcholinesterase (BChE) activity and liver damage. BChE and acetylcholinesterase (AChE) are biomarkers of low-grade systemic inflammation and earlier it was suggested that the elevation of these two bioparameters may predict development of type 2 diabetes mellitus and Alzheimer’s disease. Serum BChE activity was found to be associated with overweight, obesity, and body fat distribution parameters. Recently, a positive association between *T. gondii* seropositivity and obesity has also been reported. Infection with the parasite was linked with the increase in the number of hepatic stellate cells known to play an important role in the development of fibrosis and its advancement to cirrhosis of any etiology. Moreover, several authors suggested that hepatitis with a clinical picture resembling acute viral hepatitis result from *T. gondii* infection. Abnormalities associated with cryptogenic liver cirrhosis markedly affect acquired immunity of the host and probably participate in triggering and persistence of several autoimmune diseases, especially that anti-*T. gondii* IgG antibodies have been found in the sera of both patients suffering from these clinical entities and in healthy individuals. Oxidative stress and immunosuppression due to the infection play an important role in these processes. It should be also noted that mammalian as well as the parasite cells, express two cholesteryl ester-synthesizing enzymes, ACAT1 and ACAT2, which share 44-47% of amino acid homology. ACAT1 is present in various cells and tissues, while ACAT2 expression is restricted to hepatocytes and intestinal mucosal cells. The parasite enzymes *Tg*ACAT1 and *Tg*ACAT2 localized to endoplasmic reticulum can synthesize and store abundant esters of cholesterol and triglycerides. The lifelong persistence and resulting surplus of ACAT1 and ACAT2, and *Tg*ACAT1 and *Tg*ACAT2 enzymatic activities especially in hepatocytes and/or intestinal cells of the host may therefore at least in part be responsible for development of steatohepatitis, and generation of ballooning hepatocytes, foamy macrophages, and clear cells (foamy) colitis. These abnormalities can be explained by the suppressed autophagy (it regulates lipid metabolism) in the liver due to proliferation of *T. gondii*. In alcoholic steatohepatitis, ballooning degeneration of hepatocytes may also at least partly result from the fact that ethanol dose-dependently stimulate microneme secretion in *T. gondii* tachyzoites and parasite attachment to the host cells, thus facilitating infection of the hepatic cells. The increased serum iron levels and hepatic iron overload reported in the patients with nonalcoholic hepatitis may be caused by the excess of NO produced by activated macrophages and hepatic cells of the host, as a defense molecule against infection with the parasite. NO intercepts iron before incorporation into ferritin and directly mobilizes iron from the serum protein in a glutathione-dependent manner. Finally, several reports provided data suggesting that the generation of Mallory-Denk bodies is linked with latent chronic hepatic toxoplasmosis, and...
**INTRODUCTION**

**T. gondii** is the most prevalent chronic parasitic infection affecting about 6 billion people[^1-2] and suspected to be a global health threat[^3-4]. At present, it is believed that the parasite causes asymptomatic infection in healthy persons, but severe clinical presentations can be found in congenital toxoplasmosis, ocular involvement, and in immunocompromised individuals, including patients with AIDS[^5-6]. The purpose of this work was to present and analyze available literature data indicating a possible link between acute and chronic infection with the parasite and development of unexplained liver damage, cryptogenic liver cirrhosis, steatohepatitis, and several other hepatic histopathological abnormalities of unknown etiology.

**LIVER DAMAGE**

In animals, experimental *T. gondii* infection was associated with liver and kidney injury manifesting with a significant increase in serum alanine (AlAT) and aspartate aminotransferase (AspAT) activities, blood urea nitrogen level, decreased serum albumin concentration and alkaline phosphate activity, albumin to globulin ratio, and marked abnormalities in plasma triglyceride and cholesterol levels[^7-9]. Histopathology and histochemistry studies showed liver cells degeneration which progressed from hydropic to fatty, and marked cellular infiltration (granuloma) around the blood vessels[^10-11], the reticuloendothelial cells, Kupffer cells and phagocytes exhibited increased alkaline phosphate activity[^12]. In mice, treatment with azithromycin (a macrolide antibiotic with antitoxoplasmatic effects[^13-14]) caused normalization of serum AlAT and AspAT activities and improvement in total serum protein, albumin and globulin concentrations, and albumin/globulin ratio[^15] (Table 1-3).

It must be noted that acute *T. gondii* infection in mice caused marked reduction in serum BChE activity (*p < 0.01*) and liver damage, and positive significant correlations between BChE activity in serum and BChE activity in liver (*r = 0.89*[^16]). In addition, serum albumin concentrations were markedly decreased (Table 1), and previously it was reported that this biomarker is strongly correlated with BChE activity[^17]. At necropsy, liver of the infected animals showed random necrosis foci combined with the presence of tachyzoites and cysts containing bradyzoites, as well as an increase in spleen size. These findings are very important because AChE and BChE are biomarkers of low-grade systemic inflammation[^18-20], and it was suggested that the elevation of these two bioparameters may predict the development of type 2 diabetes mellitus and Alzheimer’s disease[^21]. These proposed associations are in line with my previous reports suggesting an important role of *T. gondii* infection in development of these two clinical entities[^22-24]. BChE also serves as a marker predicting the prognosis of diseases[^25-26] because when the serum activity of the enzyme is low, a high-risk of death must be.

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[^2]: Prandota J. Toxoplasmosis causes liver damage and cirrhosis

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**Table 1** Serum markers of liver and kidney injury in BALB/c mice infected intraperitoneally with tachyzoites of *T. gondii* (RH strain) and examined after 5 days (acc. to Da Silva et al [9]; with own modification).

<table>
<thead>
<tr>
<th>Bioparameters</th>
<th>Controls (n = 10)</th>
<th>Infected animals (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlAT (U/L)</td>
<td>35.21 ± 11.73</td>
<td>258.13 ± 84.37 *</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>538.44 ± 120.12</td>
<td>190.87 ± 78.66 *</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.21 ± 0.03</td>
<td>1.51 ± 0.08 *</td>
</tr>
<tr>
<td>Total proteins (g/dL)</td>
<td>5.80 ± 0.40</td>
<td>4.55 ± 0.44 *</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>69.28 ± 3.10</td>
<td>100.12 ± 25.75 *</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.96 ± 0.32</td>
<td>8.87 ± 0.24</td>
</tr>
</tbody>
</table>

Results represent means ± SD. *Statistically significant results compared with controls (p < 0.05).* AlAT, alanine aminotransferase.

**Table 2** Damage of liver function in mice intraperitoneally infected with 5 x 10⁹ *T. gondii* tachyzoites and treated with azithromycin (250 mg/kg/day for 3 days, p.o.) (acc.to Al-Kaysi et al [7]; with own modification).

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>Total serum protein concn (g/dL)</th>
<th>Serum albumin concn (g/dL)</th>
<th>AlAT (U/L)</th>
<th>AspAT (U/L)</th>
<th>Serum globulin concn (g/dL)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>7.42 ± 0.09</td>
<td>9.6 ± 0.26</td>
<td>10.6</td>
<td>2.56 ± 1.89</td>
<td>1.89</td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>11.02 ± 0.26</td>
<td>5.30 ± 0.26</td>
<td>59.02 ± 24.20</td>
<td>7.52 ± 0.42</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td>Infected and treated</td>
<td>8.38 ± 0.26</td>
<td>3.8 ± 0.26</td>
<td>10.6</td>
<td>4.58 ± 0.83</td>
<td>0.10</td>
<td>0.12</td>
</tr>
<tr>
<td>Not infected and treated</td>
<td>7.44 ± 0.26</td>
<td>4.88 ± 0.26</td>
<td>9.2</td>
<td>5.26 ± 1.96</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

The values are means ± SE of 10 animals per group. *Statistically significant differences between respective groups were calculated using ANOVA test at p < 0.05. A/G, serum albumin to globulin concentration ratio.

**Table 3** Serum biochemical markers of liver and kidney damage in ewes and does with toxoplasmosis (positive serum IgM antibodies directed against *T. gondii*) (acc. to Mahboub et al [8]; with own modification).

<table>
<thead>
<tr>
<th>Bioparameters</th>
<th>Sheep with <em>T. gondii</em> infection (n = 15)</th>
<th>Goats with <em>T. gondii</em> infection (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dL)</td>
<td>4.20 ± 0.15</td>
<td>4.10 ± 0.37</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>1.81 ± 0.20</td>
<td>1.24 ± 0.13 *</td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>2.39 ± 0.07</td>
<td>3.11 ± 0.28 *</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>0.85 ± 0.08</td>
<td>0.41 ± 0.087 *</td>
</tr>
<tr>
<td>AspAT (U/mL)</td>
<td>98.87 ± 1.92</td>
<td>33.2 ± 2.69</td>
</tr>
<tr>
<td>AlAT (U/mL)</td>
<td>15.11 ± 0.43</td>
<td>22.86 ± 1.99 *</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>24.33 ± 2.07</td>
<td>32.26 ± 2.56</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>17.82 ± 1.29</td>
<td>51.49 ± 6.65</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>41.61 ± 4.33</td>
<td>57.84 ± 5.17 *</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>8.40 ± 1.70</td>
<td>10.71 ± 2.48</td>
</tr>
</tbody>
</table>

Results are means ± SE. *Statistically significant results compared with respective control values (p < 0.05).* AspAT, aspartate aminotransferase; AlAT, alanine aminotransferase; BUN, blood urea nitrogen. CRP, C-reactive protein.
Prandota J. Toxoplasmosis causes liver damage and cirrhosis

taken into consideration[11,17]. The individuals with the lowest serum BChE activity had significantly higher mortality than those with the highest value[18]. In middle-aged and elderly men and women, Calderon-Margalit et al[19] showed that serum BChE activity was inversely related to age and was positively correlated with the serum levels of albumin (r = 0.35, < 0.001), cholesterol (r = 0.31, < 0.001), and triglycerides (r = 0.30, < 0.001). Moreover, the enzyme activity was strongly associated with overweight, obesity, and body fat distribution parameters (e.g., body mass index, r = 0.20, < 0.001), and recently, a positive association between T. gondii seropositivity and obesity has been found[19].

Cholinesterases are enzymes present in cholinergic and noncholinergic tissues, as well as in blood and other body fluids. The AChE is a membrane-bound enzyme mainly found in the brain, muscles, erythrocytes, lymphocytes, and cholinergic neurons[19,20], while BChE is present in the intestine, liver, kidney, heart, lung, brain, and serum[21,22]. AChE inhibits activation of macrophages and release of proinflammatory cytokines, such as IL-6, TNF-α, IL-1, and IL-18[23]. In rats, T. gondii infection was associated with a significant increase in the activity of AChE in whole blood and lymphocytes, and a positive correlation was found between the enzyme activity and number of lymphocytes (p < 0.01)[20].
The BChE is responsible for cholinergic neurotransmission[24], immune responses[25], cardiovascular risk and lipid metabolism[26]. Autonomic system dysfunctions, characterized by lower vagal and higher sympathetic tone have been documented in chronic inflammatory diseases and aging, associated certain level of systemic inflammation[25-28]. A correlation between higher vagal tone as determined by the increased frequency component of heart rate variability and lower proinflammatory cytokine levels has been reported[27,28]. Cholinergic modalities, acting through vagus nerve and/or q7 subunit-containing nicotinic acetylcholine receptor-mediated mechanisms have been shown to suppress excessive inflammation in several experimental models of disease[27,28]. In this context, numerous comorbidities frequently observed in the patients with autoimmune diseases, associated with viral and other infectious agents, including T. gondii, may be at least in part explained by the liver damage caused by the parasite[29]. The hepatic injury probably establishes a favorable local tissue and systemic environment in the body that facilitates growth and proliferation of these infectious agents further intensified by the impaired cholinergic modulation of systemic inflammation. In addition, such patients are usually subjected to many invasive procedures, including endoscopy, surgery, dental care, otorhinolaryngologic investigation, and frequently receive intravenous medications, fluids, blood and/or its components, that all increase risk of viral, bacterial and/or parasite infections[29,30].

Several authors reported that hepatitis with a clinical picture resembling acute viral hepatitis may result from T. gondii infection[21-23]. T cell function in patients with Australia antigen (AA)-associated hepatitis have shown that persistence of this antigen was related to impaired PHA-induced lymphocyte transformation[24,31]. It was suggested that viral hepatitis appeared unlikely to be the sole causal agent in primary biliary cirrhosis, because AA has been demonstrated in the serum of only 15-20% of patients[18,32]. Because development and clinical course of these comorbidities are often associated with an increased production of various pro- and anti-inflammatory cytokines which are overlapping with T1 and T2 type of cytokines generated by T.gondii infection, such an excess of various factors and released mediators may exert beneficial or harmful effects in the host, depending on their final constellations in the body. These suggestions are in agreement with recent reports that emphasized an important role of the parasite in development of liver damage and T. gondii-related hepatitis[39-41].

The above-presented data suggest that liver damage caused by latent chronic T. gondii infection has an important impact on the innate and acquired immunity of the host, development of various comorbidities and their clinical course, and finally on improvement/ worsening of the host’s health. The seroprevalence of anti-T. gondii IgG antibodies in various autoimmune diseases, including primary biliary cirrhosis have been presented in Tables 4 and 5. In these patients, impaired cellular immunity due to the infection with the parasite may also play an important role in triggering, development and persistence of these clinical entities[32].

### CRYPTOGENIC LIVER CIRRHOSIS (CLC)

This chronic liver disease with so far not established particular etiology accounts for 5-30% of cases of cirrhosis and about 10% of liver transplants[42-45]. In Germany, presumed cryptogenic liver disease (probably an early phase of liver cirrhosis) was found to be associated with high prevalence of autoantibody-negative autoimmune hepatitis, low prevalence of nonalcoholic steatohepatitis (NASH), and no evidence for occult viral etiology[40]. At present, it is believed that the leading causes of CLC include previously unrecognized NASH, silent autoimmune hepatitis, occult

### Table 4 Serum IgG levels of anti-infectious agents antibodies more prevalent in primary biliary cirrhosis compared with controls (acc. to Shapira et al[42]; with own modification)

<table>
<thead>
<tr>
<th>Infectious agent</th>
<th>Patients with primary biliary cirrhosis</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. gondii (IU/mL)</td>
<td>53.4 ± 9.1</td>
<td>36.4 ± 7.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>EBV-EA AI</td>
<td>1.7 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

AI, antibody index. EBV-EA, Epstein-Barr virus - early antigen. Results are given as mean ± SE. Anti-EBV-EA antibodies concentrations positively correlated with anti-T. gondii antibody levels in those patients (Spearman correlation r = 0.35, p < 0.01). A similar positive correlation was detected in PBC patients between anti-EBV-EA and CMV antibodies levels (r = 0.32, p < 0.01), which may suggest a secondary origin of their generation as compared with the primary and the same T. gondii-related IL-21 cytokine stimulus intensity required to control chronic viral infection[43-45].

### Table 5 Prevalence of anti-T. gondii IgG antibodies in 1514 serum samples of patients with various autoimmune diseases (acc. to Shapira et al[46]; with own modification)

<table>
<thead>
<tr>
<th>Disease/Clinal entity</th>
<th>Anti-T. gondii IgG positive</th>
<th>Geographical region</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiphospholipid syndrome (APS)</td>
<td>82/159 (52%)</td>
<td>Europe</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Cryptoglobulinemia</td>
<td>65/117 (56%)</td>
<td>Europe</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>ANCA-associated vasculitis</td>
<td>45/68 (66%)</td>
<td>Europe</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Autoimmune thyroid diseases</td>
<td>69/120 (57%)</td>
<td>Europe</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Systemic sclerosis</td>
<td>46/80 (58%)</td>
<td>Europe</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>27/35 (77%)</td>
<td>Europe</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>55/152 (36%)</td>
<td>Latin America</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Including primary and secondary APS; † Including cryoglobulinemia and mixed cryoglobulinemia. ‡ P values for the comparison with matched healthy controls of the same geographical region. In addition, the following specific autoimmune diseases were associated with markedly increased serum anti-T. gondii IgG antibodies/autoantibodies: polymyositis[47], rheumatoid arthritis[48,50], Hashimoto’s and Graves’ diseases[51,52], Cohn disease[50,53], antiphospholipid syndrome[54], Wegener’s granulomatosis and other vasculitides[55], autoimmune biliary disease [56], systemic lupus erythematosus[57], diabetes mellitus type 1 and 2[6,53,58], optical nerve and retinal diseases with visual loss (the 22 kDa neuronal antigen found in the patients may represent T. gondii antigen[59-61].
non-B, non-C viral hepatitis (hepatitis X), and occult past ethanol exposure\cite{60}. Leite et al\cite{61} reported that in their 47 patients with CLC, the following prevalences were demonstrated: impaired fasting glycemia (68\%), low HDL levels (58.1\%), total hypercholesterolemia (27.9\%), hypertriglyceridemia (16.3\%), and obesity (27.5\%). The results obtained in the patients with CLC showed statistical similarity with the data of the NASH group regarding fasting glycemia (62.8\%) and HDL levels (53.8\%, males)\cite{83}. Caldwell et al\cite{84} found that among their 70 consecutive patients with cryptogenic cirrhosis, both diabetes type 2 and obesity (73\% of individuals) were significantly more common compared with the cirrhotic patients with primary biliary cirrhosis or hepatitis C. It must be noted that among 567 patients with chronic liver disease evaluated by Kodali et al\cite{85}, 28 individuals (4.9\%) had no established a definite etiology, and nearly half of the patients with presumed CLC (13 of them, 46\%) had a history of previous blood transfusion, thus supporting the hypothesis of a non-A, non-B, and non-C hepatitis virus. This may suggest that latent \textit{T. gondii} infection associated with blood transfusion plays at least in part an important role in development of cryptogenic liver disease, and support the indication of several authors for screening blood donors for serum AIAT as well as \textit{T. gondii} IgG activities to reduce posttransfusion hepatitis\cite{13,14,15,16,17}. Moreover, it was found that the infection with the parasite significantly affected glycolysis, gluconeogenesis and tricarboxylic acid cycle metabolic processes\cite{91}, and probably was responsible for development of type 1 and 2 diabetes, and obesity\cite{92,93,94}. These findings are therefore well in line with the suggestion that cryptogenic liver disease in children represents a novel congenital disorder of glycosylation\cite{95}.

In a study of 354 patients with unexplained abnormal liver function tests and in the absence of diagnostic serology, liver biopsy showed some degree of fibrosis in 26\% of individuals, and 6\% were cirrhotic\cite{97}. Thirty four and 32\% of biopsies suggested NASH or fatty liver, respectively. Other diagnoses included cryptogenic hepatitis, drug toxicity, secondary biliary cirrhosis, and autoimmune hepatitis\cite{98}. NASH was considered the commonest cause of cryptogenic cirrhosis\cite{99,100}, also associated with diabetes and obesity in some individuals\cite{101}. In two other studies, cirrhosis of unknown etiology was found in 3-30\% of individuals hospitalized because of this clinical entity\cite{102,103}. The so-called residual histological findings, such as foci of autoimmune-like inflammatory infiltrates versus NASH-like foci of steatosis, cellular ballooning, and glycogenated nuclei may finally help in defining the underlying cause of cryptogenic cirrhosis\cite{104,105}.

Interestingly, subclinical liver involvement is frequent in systemic lupus erythematosus (SLE), Cowdhary et al\cite{106} identified 40 SLE patients with liver enzyme abnormalities, and biopsies performed in 20 individuals showed changes characteristic for nonalcoholic fatty liver disease ($n = 8$), autoimmune hepatitis (6), primary biliary cirrhosis (3), hepatitis C (3), and cryptogenic cirrhosis (2). Systemic sclerosis (SSc) is another autoimmune disease characterized by vascular obliteratiion, excessive extracellular matrix deposition, and fibrosis of the connective tissues of the skin, lung, gastrointestinal tract, heart, and kidneys. Endothelial cell dysfunction and damage represent one of the first disturbances in the pathogenesis of SSc, and chronic inflammation plays an important role in the development of atherosclerotic plaques\cite{107}. A 60-kDa protein belonging to the heat shock proteins (HSPs) was suggested to be involved in the pathomechanism of these abnormalities\cite{108}. It was demonstrated that Hsp60 bound lipopolysaccharide (LPS) tightly and it was the Hsp60-bound LPS, not Hsp60 itself, that was responsible for the observed cytokine effects of Hsp60 preparations\cite{109}. In addition, Hsp60-bound LPS was more potent than LPS alone in inducing cytokine production\cite{110,111}. It must be noted that \textit{T. gondii} HSPs, particularly Hsp60 and Hsp70 (Hsp70 provides cellular protection against oxidative stress and functions as a B cell mitogen) released mainly during bradyzoite transition are highly immunogenic, capable of inducing antibody production and T-cell activation\cite{112,113}. Each of these HSPs was found to bound a distinct set of peptides, including antigen peptides and its precursors\cite{114}, and various HSPs have different efficiency to activate monocytes and dendritic cells\cite{115}, as well as to release NF-$\kappa$B, NO and other pro- and anti-inflammatory cytokines\cite{116,117,118}. Specifically, Hsp60 exerted immune stimulatory effects on vascular endothelium (upregulation of adhesion molecules), smooth muscle cells (IL-6)\cite{119}, monocyte/macrophages inducing NO, IL-6, IL-12 and TNF-$\alpha$ production\cite{120,121,122}, dendritic cells (IL-1$\beta$, IL-6, IL-12, TNF-$\alpha$ maturation)\cite{123,124}, B cells (proliferation, IL-6, IL-10, upregulation of MHCII, CD69, CD40, CD86)\cite{125}. In this context, it should be emphasized that the proteins with molecular weights of 58 and 60 kDa have been demonstrated amongst excretory/secretory protein antigens released from purified tachyzoites/bradyzoites of \textit{T. gondii} and various components of the parasite in samples of human and animal sera\cite{126}.

Tweezer-Zaks et al\cite{127} found nine patients with cryptogenic cirrhosis comprising 0.15\% of the familial Mediterranean fever (FMF) patient population. This rate was significantly higher than the rate of 0.015\% of cirrhosis of all types expected in the total population of Israel ($p < 0.000$). FMF is a disorder presenting in childhood or adolescence with 1- to 3-day episodes of fever often accompanied by severe abdominal pain, pleurisy, monoarticular arthritis, or an erythematous rash on the ankle or foot known as erysipeloid erythema\cite{128}. Importantly, targeted disruption of pyrin, the FMF protein, caused heightened sensitivity to endotoxin and a defect in macrophage apoptosis\cite{129}. In addition, pyrin appeared to have both inhibitory and potentiating effects on IL-1$\beta$ production and played a role in regulating NF-$\kappa$B activation and apoptosis\cite{130,131}. It was suggested that in patients with FMF \textit{T. gondii} infection plays an important role in the pathogenesis of Mollaret meningitis\cite{132}. Moreover, in human astrogliosis cells and in murine mast cells infected with \textit{T. gondii} tachyzoites, the parasite induced upregulation of profibrotic factors, such as matrix metalloproteinase-2 and -9 (MMPs) via Erk1/2-NF-$\kappa$B pathway\cite{133}, and MMPs are a family of the extracellular matrix-degrading enzymes that are generally synthesized and secreted as latent soluble enzymes requiring activation in the extracellular space\cite{134}.

In summary, these data suggest that latent chronic \textit{T. gondii} infection may be responsible for development of CLC. Abnormalities associated with CLC markedly affect acquired immunity of the host and probably participate in triggering and maintaining several autoimmune diseases, especially that anti- \textit{T. gondii} IgG antibodies have been found in the sera of both patients suffering from many of these clinical entities and in healthy individuals\cite{135,136}, which is in line with a cosmopolitan distribution of the pathogen.

**MOLECULAR PATHOBIOLOGY OF HEPATIC TIBROSIS**

Chronic inflammatory response is sometimes associated with the accumulation of extracellular matrix components including collagens, fibronectin and hyaluronic acid, and numerous fibrotic diseases affecting the liver, lung, skin, heart, and kidney, are believed to have an infectious etiology with bacteria, viruses, \textit{T. gondii}, and fungi\cite{137}. Also, hepatic fibrosis may be associated with autoimmune liver disorders, including autoimmune hepatitis, primary biliary cirrhosis.
or primary sclerosing cholangitis. Chemokines, such as for example, a profibrotic factor CXCL 10, promote hepatic inflammation and liver cirrhosis by prevention of NK cell mediated hepatic stellate cells (HSCs) inactivation, because the cytokine is responsible for the recruitment and localization of inflammatory cells to sites of tissue damage or infection[112]. It must be noted that HSCs secrete CXCL 10 when stimulated with IFN-γ[112], and during T. gondii infection the cell numbers markedly increased[40]. Moreover, Furtado et al[40] found that human monocytic-derived dendritic cells infected with T. gondii tachyzoites transmigrated in larger numbers across stimulated human retinal endothelium than noninfected dendritic cells (p < 0.004). Antibody blockade of ICAM-1, VCAM-1, and activated leukocyte cell adhesion molecule inhibited transmigration, while chemokines CCL21 or CXCL 10 increased this process[113].

Ustun et al[114] investigated etiology of liver cirrhosis and frequency of T. gondii seroprevalence in 108 patients and found that toxoplasma IgG and IgM seropositivity was present in 74 (68.5%) individuals compared with 24 (48%) of 50 participants in the control group (p < 0.05) (Tables 6 and 7). A case-control epidemiological study demonstrated a link between T. gondii infection with liver cirrhosis[110], Atmaca et al[40] showed that infection with the parasite in mice was associated with the increase in the number of HSCs. The number of these cells was highest on day 6 after inoculation of animals as compared with the numbers found on days 4 and 2 after inoculation, and in controls (all p < 0.05). There was also a significant relationship between the number of GFAP-positive HSCs when they were compared with T. gondii antigen immunostaining (p < 0.05), and the amount of immunostaining markedly increased with the increase in the number of HSCs[40] (Table 8). HSCs are known to play an important role in development of fibrosis and its advancement to cirrhosis as indicated by their close proximity to collagen fibers in injured livers[118], and finding as the main matrix-producing cells in the process of liver injury and fibrosis of any etiology[41]. In the activation process of these cells, TGF-β was believed to be the main mediator of fibrogenesis, while platelet-derived growth factor (PDGF) was the major inducer of HSCs proliferation[117-119]. Also leptin has been reported as a novel profibrogenic cytokine in HSCs[120]. [Nb. in rats T. gondii infection caused a significant increase of plasma leptin concentration (p < 0.01), with no change in body weight of animals[211], in obese individuals, leptin exerted a proinflammatory activity, and its structure is similar to that of IL-2, an important proinflammatory cytokine (Table 9)].

Activated HSCs differentiate from vitamin-A-storing cells to contractile myofibroblastic cells responsible for extracellular matrix production in the damaged liver[118]. HSCs may also interact with the sinusoidal space, thus affecting the function of the antigen presenting cells, such as dendritic cells, liver macrophages or Kupffer cells[128]. It must be noted that in several patients HSCs activation and tissue inhibitor of metalloproteinase overexpression persisted even after eradication of the underlying known cause of liver injury, preventing resolution of fibrosis, as for example HCV infection[127,129]. This observation is in a full agreement with the recent case-control study demonstrating that high serum anti- T. gondii IgG concentrations significantly correlated with high HCV load levels among chronic hepatitis C patients (p = 0.026)[129], and with my reasoning suggesting an important role of chronic latent T. gondii infection in several such unresolved cases, especially that both PDGF and TGF-β also have been reported to play an important role in the pathobiology of the parasite[129-132]. For example, T. gondii increased production of TGF-β, activated hypoxia-inducible factor 1 (HIF1) already at physiologically relevant oxygen levels, and required HIF1 for growth and survival[133,134]. The human platelet contribution against Table 9 Serum proinflammatory cytokines and leptin concentrations in obese children at prepubertal age compared with healthy children of the same age (acc. to Aygun et al.[122] and Kapiotis et al.[123], with own modification)
the intracellular growth of *T. gondii in vitro* in human pulmonary fibroblasts showed that tachyzoites of the parasite induced activation of human platelets, and PDGF mediated inhibition of the intracellular growth in a virulent *T. gondii strain*. It was found that human platelet to the parasite ratios as low as 1:3 were toxic to the protozoan with distinct cell-cell contact essential for platelet-mediated cytoxicity. Adherence of platelets to *T. gondii* and disruption of surface membranes and cytoplasmic contents of the organisms were documented ultrastructurally. Thromboxane (TX) played an important role in these processes because both the TXA₂-generating platelet microsome system and a stable TXA₂ analog-induced damage to the cellular membranes of the parasite, as noted by transmission electron microscopy. Recently, Henderson et al. showed that *T. gondii*-stimulated platelets released oxygenated products of both (13-hydroxyoctadecadienoic acid and no acid - 12.3%), that also may be of importance for the cytotoxic host defense response against infection with the parasite.

**8ALLOONED HEPATOCYTES AND MALLORY-DENK BODIES (MDBs)**

Hepatocellular “ballooning” is an important histological hallmark of liver pathology found particularly in steatohepatitis manifested by degeneration of hepatocytes associated with enlargement, swelling, rounding, and characteristic reticulated cytoplasm. These changes in hepatocyte morphology may be observed also in many other acute and chronic liver diseases, including autoimmune hepatitis, neonatal giant hepatitis, and alcoholic liver disease, both alcoholic and nonalcoholic steatohepatitis, and ischemia/reperfusion injury of liver allografts. The intermediate keratin filaments (IF) play an important role in the stabilization and topographical localization of a cell and its organelles, and a pronounced diminution or even loss of keratin 8/18 was found in the IF cytoskeleton of ballooned hepatocytes. MDBs are an intracellular deposition of misfolded proteins (keratins, chaperones Hsp90 and 25 families, α-B crystallin, phosphoepitopes/kinases, protein degradation machinery, and others, such as ubiquitin, tubulin, as well as intracytoplasmic hyaline inclusions, glycogen bodies, ground glass inclusions (hallmarks of chronic HBV infection reflecting hypertrophic endoplasmic reticulum), pale bodies, α-antitrypsin inclusions and megamitochondrial. Sometimes MDBs appear as numerous small globular structures distributed throughout the cytoplasm, near nucleus, or localized at the cell periphery (early MDBs = small cytoplasmic globular structures; mature MDBs = large paranuclear inclusions; old MDBs = involution stage present at the cell periphery). Interestingly, IF cytoskeleton damage was shown to result from oxidative stress in various human liver diseases and in the mouse models of steatohepatitis. It seems that *T. gondii* infection may be at least partly responsible for development of these abnormalities because Yang et al. showed that in mice injected intraperitoneally with *T. gondii* tachyzoites, the serum level of the oxygen free radicals (NO, “OH, O₂”) increased along with the days of infection, and superoxide dismutase concentration reached a peak on the 3rd day of the investigation. In asymptomatic *T. gondii*-seropositive blood donors oxidative stress and immunosuppression have also been demonstrated. Moreover, it was found that oxidative stress with markedly increased serum malondialdehyde and decreased glutathione levels is characteristic for *T. gondii* seropositive patients and chickens orally infected with tissue cysts of the protozoan (Tables 10 and 11).

Hepatic steatosis is caused by the abnormal accumulation of predominantly triglycerides in the liver. Histological examinations of liver parenchyma revealed presence of triglyceride droplets in the cytoplasm of hepatocytes and their accumulation in small and large vesicles which impairs liver function and makes this organ more susceptible to other injuries. Immunochemical analysis of steatohepatitis performed by Lackner et al. showed that in macrovesicular steatosis the IF cytoskeleton was only pushed to the cell periphery by the accumulated triglycerides, as it was also observed in all their cases of steatosis. In alcoholic steatohepatitis, ballooning degeneration of hepatocytes can at least partly be explained by the finding that ethanol and its metabolite acetaldehyde elevated intracellular concentration of Ca²⁺ by mobilizing the ion from intracellular stores. Moreover, ethanol dose-dependently stimulated micromere secretion in *T. gondii* tachyzoites and parasite apical attachment to host cells, thus facilitating infection of hepatic cells. Ethanol is known to have several targets in a variety of vertebrate cells by affecting signal transduction, such as for example activation of phosphatidylinositol-specific phospholipase C in hepatocytes, and triggered amylase secretion from pancreatic acini by increasing Ca²⁺ levels, and therefore together with oxidative stress could be the cause of marked disturbances in function and morphology of the host cells. Furthermore, liver microsomal fatty acid oxidizing enzymes, such as cytochrome P450 CYP2E1 expression are increased in both alcoholic fatty liver disease (AFLD) and nonalcoholic fatty liver disease (NAFLD). Since CYP2E1 also metabolizes ethanol, fatty acid-related induction of this enzyme contributes to generation of acetaldehyde, which forms immunogenic adducts with various intra- and extracellular biomolecules and exacerbates reactive oxygen species production in AFLD. In this context, the prevalence of non-organ-specific autoantibodies (NOSAs) and chronic liver disease in general population, and NOSAs as the hallmark of a subclinical autoimmune disease (AFLD), the exacerbation of autoimmune chronic hepatitis by interferon therapy, the presence of autoantibodies to cytoskeletal filaments, actin and intermediate filaments in chronic active hepatitis, as well as the evidence against hepatitis viruses as important causes.

### Table 10 Levels of malondialdehyde (MDA) and glutathione (GSH) in heart and aorta of broiler chickens infected intraperitoneal with 50 tissue cysts of *T. gondii* (acc. to Al-Kennany et al.[47], with own modification).

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol per gm wet tissue)</th>
<th>GSH (nmol per gm wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>125 ± 1.05</td>
<td>105 ± 0.33</td>
</tr>
<tr>
<td>Infected</td>
<td>329.8 ± 2.32 *</td>
<td>453.3 ± 0.43 *</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SD of 10 broiler chickens per group. *Significant differences at p ≤ 0.05.

### Table 11 Serum glutathione, malondialdehyde, and NO concentrations in *T. gondii*-seropositive patients and healthy controls (acc. to Karaman et al[60], with own modification).

<table>
<thead>
<tr>
<th>Bioparameter</th>
<th>Group</th>
<th>No of participants</th>
<th>Serum levels (mean ± SD)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione</td>
<td>Patients</td>
<td>37</td>
<td>3.96 ± 0.10</td>
<td>0.001</td>
</tr>
<tr>
<td>Controls</td>
<td>40</td>
<td>10.37 ± 0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malondialdehyde</td>
<td>Patients</td>
<td>37</td>
<td>41.32 ± 2.05</td>
<td>0.001</td>
</tr>
<tr>
<td>Controls</td>
<td>40</td>
<td>9.18 ± 1.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>Patients</td>
<td>37</td>
<td>47.47 ± 1.00</td>
<td>0.001</td>
</tr>
<tr>
<td>Controls</td>
<td>40</td>
<td>59.18 ± 1.29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Serum glutathione and NO levels are expressed as µmol/L, and malondialdehyde concentrations represent nmol/L. Results statistically significant at p < 0.05.
of autoimmune hepatitis\textsuperscript{[161]}, are all well in line with worldwide dissemination of the parasite and modulaton of the host cell proteome by the intracellular pathogen (e.g. cytoskeleton-associated protein 4, actin filament, actin beta, actin gamma 1 propeptide, actinin-1 alpha, etc.\textsuperscript{[179]})\textsuperscript{[179]}. A similar profuse generation of antibodies and autoantibodies directed against brain proteins in patients with autism and their families probably associated with \textit{T. gondii} infection\textsuperscript{[160]} also seems to further support the above-presented reasoning. Studies in animals showed that oral infection with \textit{T. gondii} resulted in different quantitative and morphologic changes in myenteric plexus neurons. For instance, in rats no changes in the population or density of these cells were observed\textsuperscript{[162,163]}, while there was an intense myenteric plexus neuronal cells death and atrophy reported in chicken\textsuperscript{[164]}. On the other hand, in pigs orally infected for 30 days with oocysts of \textit{T. gondii} (M7741 strain) Odorizzi et al.\textsuperscript{[165]} showed that the number of nitricergic NADPHd-p neurons per ganglion markedly increased, and the cells became hypertrophic through the augmentation of the cell body by 12.8% (\(p < 0.0001\)), and specifically through the increase of the nuclear area by 24.8% (\(p < 0.0001\)) (no change was observed in the distribution among different classes of neuronal cell sizes) (Tables 12 and 13). Because NO play an important role in resistance of the host cells against \textit{T. gondii} infection\textsuperscript{[166,167]}. It was suggested\textsuperscript{[165]} that the cell hypertrophy may be indicative of the increased generation of biochemical mediator(s) with antiparasitic activity that may simultaneously exert a detrimental effect on the tissue cells containing parasites\textsuperscript{[164,165]}. This reasoning is in line with the above argumentation that the oxidative stress associated with \textit{T. gondii} infection play an important role in development of disturbances of the host cell morphology. Finally, one may suggest that MDBs and the intracellular hyaline bodies observed in neoplastic and nonneoplastic hepatocytes\textsuperscript{[162,164]} are linked with latent chronic intracellular \textit{T. gondii} infection. These peculiar abnormalities probably represent the remnants of tachyzoites/bradyzoites/sporozoites/oocysts, such as for example amylopectin, lipid droplets, PV membranes, etc., biotransformed by changing (especially acidic) pH of the inflamed liver tissue and various enzymes of both the host cell and the parasite itself, like it was suggested for the amyloid plaques produced in the brain cells of patients with Alzheimer’s disease\textsuperscript{[161]}. Interestingly, Mayer et al.\textsuperscript{[168]} also suggested that MDBs resemble inclusion bodies associated with chronic neurodegenerative diseases, including Alzheimer’s and Parkinson’s diseases.

### STEATOHEPATITIS

\textit{T. gondii} is a sterol-auxotrophic organism incapable of sterol synthesis and therefore scavenges cholesterol from mammalian host cells\textsuperscript{[170]}. Similarly as in mammalian cells, most of this lipid is concentrated in the plasma membrane of the parasite\textsuperscript{[211]}. Cholesterol is a vital part of membranes but its overabundance in cells leads to inflammation and oxidative damage\textsuperscript{[179]}. Mammalian cells as well as the parasite, express two cholesterol ester (CE)-synthesizing enzymes, ACAT1 and ACAT2, which share 44-47% of amino acid homology\textsuperscript{[170,172]}. ACAT1 is a ubiquitous and is responsible for CE formation in brain, adrenal glands, macrophages, and kidneys, while ACAT2 is expressed in the hepatocyte as the major cholesterol esterifying enzyme in human liver\textsuperscript{[173]} and in the small intestine villi\textsuperscript{[174,175]}. Free cholesterol (a toxic polar lipid) is also esterified by ACAT2 to CEs (nonpolar compounds)\textsuperscript{[172]}. ACAT2 plays a pivotal role in dietary cholesterol absorption\textsuperscript{[175]}. In mammalian cells, the main stored neutral lipids are triacylglycerol and CEs, which are produced by two related enzymes, acyl-CoA: diacylglycerol acyltransferase (DGAT) and acyl-CoACholesterol acyltransferase (ACAT), respectively, and both can contribute to membrane biogenesis\textsuperscript{[176,177]}. Both enzymes ACAT1 and ACAT2 utilize cholesterol or oxysterols but different acyl-CoA (ACAT1 - 20:4; ACAT2 - 16:0,18:1,18:2)\textsuperscript{[217]}. 

\textit{T. gondii} enzymes also can synthesize and store both abundant esters of cholesterol (\textit{TgACAT1} and \textit{TgACAT2})\textsuperscript{[178,179]} and triglycerides (\textit{TgDGAT1})\textsuperscript{[179]}. Cholesterol ester oleate C18:1 (42%) and palmitate C16:0 (26%) are the main esters in mammalian cells, but the parasite also has uniquely large amounts of cholesterol eicosanolate C20:1 (7%), and lesser amounts of cholesterol palmitoleate C16:1, stearate C18:0, linoleate C18:2, arachidonate C20:4, and some polysaturated C22 fatty acids\textsuperscript{[170]}. It should be noted that both these enzymes (ACAT1 and ACAT2) are endoplasmic reticulum (ER)-resident proteins. Interestingly, the MDBs were reported to contain ground glass inclusions described as the hallmarks of chronic HBV infection reflecting hypertrophic ER\textsuperscript{[142]}, whereas ACAT2 produced CEs that were assembled just in the ER\textsuperscript{[171]}. One cannot therefore exclude that CEs accumulated in the hypertrophic ER of the patients with HBV infection\textsuperscript{[142]}, at least in part were the result of abundant \textit{TgACAT2} activity in the hepatic cells of those individuals who had a concomitant subclinical \textit{T. gondii} infection.

The fact that mammalian ACAT2 expression is restricted to hepatocytes and intestinal mucosal cells\textsuperscript{[170,174]}, is very important because it strongly supports our earlier suggestion that peroral \textit{T. gondii} infection plays an active role in the pathogenesis of “clear cell” colitis with foamy macrophages present in intestinal tissues\textsuperscript{[131]}. Moreover, the ACAT expression in the hepatocytes probably is also involved in the development of nonalcoholic steatohepatitis because \textit{TgACAT2} produces more CEs than \textit{TgACAT1} and has broader fatty

<table>
<thead>
<tr>
<th>Cell components</th>
<th>NADPH-d-p</th>
<th>NADH-d-p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CG</td>
<td>EG</td>
</tr>
<tr>
<td>Cell body area</td>
<td>633.1</td>
<td>475.0; 857.4</td>
</tr>
<tr>
<td>(µm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleus area</td>
<td>144.8</td>
<td>499.5; 207.5</td>
</tr>
<tr>
<td>(µm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleus/cell</td>
<td>0.24</td>
<td>0.017; 0.31</td>
</tr>
<tr>
<td>body area ratio</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For 30 days with oocysts of \textit{T. gondii} strain and examined 30 days post inoculation (acc. to Odorizzi et al.\textsuperscript{[165]} with own modification).
acid specificity. The parasite was competent to synthesize CEs using host cell-derived cholesterol and fatty acid, and the production was proportional to the amount of lipid taken up by the protozoan over the time. It appeared that several FFAs were incorporated into parasite cholesteryl ester, suggestive of broad fatty acid specificity, but nearly twofold higher incorporation of palmitate into cholesteryl ester as compared with oleate, arachidonate, stearate, and linoleate was observed. (Tables 15 and 16) This preferential palmitate incorporation specificity for T. gondii acyl-CoA: diacylglycerol acyltransferase (TgDGA T1) has already been found also for triacylglycerol synthesis in the parasite, and TgDGA T1 is an integral membrane protein localized to the parasite cortical and perinuclear endoplasmic reticulum. The protozoan was able to take up both oleate and diacylglycerol and incorporate them into the triacylglycerol fraction, and oleate was incorporated into diacylglycerol (Table 17).

Lige et al. suggested that the parasite scavenges LDL-cholesterol by uncontrolled uptake mechanisms, and engorges itself with cholesterol proportionally to the amount of the lipid in the environment. One may therefore speculate that this abnormality finally manifest histopathologically as steatohepatitis, and morphologically represent cholesterol ester-laden macrophages/foam cells, because the number and the size of lipid bodies in T. gondii vary between the different parasite developmental stages (tachyzoites, bradyzoites, oocysts), environmental conditions, infected cells/tissues of the host, and intensity of oxidative stress associated with the infection. Moreover, proliferation of the parasite in the liver cells suppresses host cell autophagy in that organ (as well as in other cells), leading to abnormally high levels of cholesterol along with aberrant triglycerides deposition because of defective clearance of lipid droplets.

Interestingly, the activities of ACAT1 and ACAT2 can be stimulated in mutant parasites in response to accumulated free cholesterol. Concomitant to the increase in CEs content, the number and size of lipid bodies are dramatically enhanced in mutant parasites, probably to collect excess CE, and thereby providing a “safety sink” for toxic free cholesterol. It should however be added that in healthy individuals lipid bodies found in the cytoplasm of a variety of cells, including leukocytes, macrophages, play an important role in modulating innate immune defense response.

### Table 14 Uptake of cholesterol by MEF ACAT1 and ACAT2, and incorporation into cholesteryl esters

<table>
<thead>
<tr>
<th>MEF ACAT1 transected with</th>
<th>Cholesterol uptake (cpm x mg cell protein-1)</th>
<th>Cholesterol ester levels (cpm x mg cell protein-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector</td>
<td>780 ± 99</td>
<td>1833 ± 105</td>
</tr>
<tr>
<td>TgACAT1</td>
<td>4003 ± 510</td>
<td>898 ± 65</td>
</tr>
<tr>
<td>TgACAT2</td>
<td>578 ± 436</td>
<td>1514 ± 110</td>
</tr>
</tbody>
</table>

MEF ACAT1 were transfected with vector alone, or plasmids containing either TgACAT1 or TgACAT2. Cells were then incubated with [3H] cholesterol-LDL. Cholesterol uptake was monitored by scintillation counting. Cholesterol ester detection was performed by thin-layer chromatography analysis. The results are mean values ± SD from three incubations (n = 3) statistically significant, p < 0.01. MF, mouse embryonic fibroblasts. ND, not detected. CPM, counts per minute.

### Table 15 Comparison of free fatty acids (FFAs) uptake by T. gondii and CHO cells (acc. to Quittnat et al [176], with own modification)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>T. gondii</th>
<th>CHO cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleate</td>
<td>780 ± 99</td>
<td>1220 ± 142</td>
</tr>
<tr>
<td>Palmitate</td>
<td>1833 ± 105</td>
<td>1156 ± 101</td>
</tr>
<tr>
<td>Stearate</td>
<td>810 ± 69</td>
<td>1467 ± 151</td>
</tr>
<tr>
<td>Linoleate</td>
<td>750 ± 91</td>
<td>1550 ± 98</td>
</tr>
<tr>
<td>Arachidonate</td>
<td>767 ± 77</td>
<td>1108 ± 103</td>
</tr>
</tbody>
</table>

CHA, Chinese hamster ovary. Extracellular parasites or CHO cells were incubated for 1 h at 37°C with the indicated 0.3 mM radioactive FFAs. After washing, the total FFAs uptake was determined by measuring the cell-associated radioactivity and expressed in nmol per mg cell protein/h. Results are means ± SD of four separate experiments. Differences between values of the uptake of palmitate vs. other FFAs in T. gondii are statistically significant. *p < 0.005.

### Table 16 Fatty acid specificity for triacylglyceride (TAG) synthesis in T. gondii (acc. to Quittnat et al [176], with own modification)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Relative activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>3Holeate</td>
<td>1</td>
</tr>
<tr>
<td>3Holeate + palmitate</td>
<td>0.18</td>
</tr>
<tr>
<td>3Holeate + stearate</td>
<td>0.69</td>
</tr>
<tr>
<td>3Holeate + linoleate</td>
<td>0.45</td>
</tr>
<tr>
<td>3Holeate + arachidonate</td>
<td>0.61</td>
</tr>
</tbody>
</table>

CHO, Chinese hamster ovary. Extracellular T. gondii and CHO cells were incubated for 1 h at 37°C with 10 nM of radioactive oleate (~50,000 cpm per nmol) previously mixed with 0.3 mM of the indicated unlabeled FFAs to measure its incorporation into TAG. The production of TAG with oleic acid alone was 45,950 cpm per mg cell protein for T. gondii. The values expressed as relative activities, are means of three separate experiments.

### Table 17 Lipid acquisition and substrate incorporation into n-acylglycerides of T. gondii (acc. to Quittnat et al [176], with own modification)

<table>
<thead>
<tr>
<th>Lipid uptake (cpm per mg cell protein)</th>
<th>Lipid synthesis (cpm per mg cell protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THOlate</td>
<td>78589 ± 190</td>
</tr>
<tr>
<td>TAG</td>
<td>10444 ± 1874</td>
</tr>
<tr>
<td>DAG</td>
<td>32430 ± 3860</td>
</tr>
</tbody>
</table>

DAG, diacylglycerol; TAG, triacylglycerol; cpm, counts per minute. Extracellular T. gondii were incubated for 1 h at 37°C with radioactive oleate or DAG before measurement of total lipid associated to parasites and lipid incorporation into DAG or TAG. The values are means ± SD of three separate experiments.

### HEPATIC IRON OVERLOAD IN STEATOHEPATITIS

Patients with nonalcoholic steatohepatitis and chronic viral hepatitis had increased serum iron levels and hepatic iron overload. This finding is consistent with cytotoxic actions of NO produced by activated macrophages and a model of glucose-dependent...(continued)
NO-mediated iron mobilization from cells proposed by Watts & Richardson[205]. They showed that NO intercepted iron before incorporation into ferritin and directly mobilized iron from ferritin in a glutathione-dependent manner. It should be emphasized that NO is produced in excess by the host cells as an important defense molecule directed against T. gondii infection[212,214,215]. On the other hand, iron is also necessary for multiplication of tachyzoites[212], and therefore overloading with iron is associated with persistent generation of a circulus viciosus, detrimental for the host. Moreover, the excessive iron accumulation in liver causes hepatocellular injury and fibrosis[196,203], because reactive iron species produced in hepatocytes result in iron-dependent microsomal peroxidation of polyunsaturated fatty acids[199,204] and lipocyte activation/lipocyte fibrogenesis[203,203]. Similar abnormalities in iron and copper metabolism play a crucial role also in the pathogenesis of various neurodegenerative diseases[203,204], and this may further support an important role of T. gondii infection in development of these disturbances.

**FOAMY MACROPHAGES AND FOAMY COLITIS**

Characteristic foamy macrophages demonstrated in histopathological specimens are an important diagnostic factor in foamy colitis, and confirm mild and moderate course of that type of inflammation in children[200-209]. The induction of foamy macrophages packed with lipid bodies have been reported in several clinical pathologic states associated with chronic proinflammatory stimuli, including T. gondii[208] and another intracellular human pathogen, Mycobacterium tuberculosis infections[239]. Development of foamy colitis (microscopic colitis)[205] may be due to oral infection with T. gondii. The parasite employs host low-density lipoproteins (LDL) receptor to acquire cholesterol[207] and diverts it for cholesteryl ester synthesis and storage in lipid bodies[178,193,219], leading to the formation of foam cells[208]. Macrophages convert into foam cells through a dysregulation in the balance between the influx and efflux of LDL particles (containing cholesterol, triacylglycerides and phospholipids) from the serum. Foamy macrophages are not only the product of an inflammatory response but amplify that response through production of PGE₂ and leukotrienes[211,212], and appeared to be a key player in both sustaining persistent bacteria and contributing to development of human tuberculosis granuloma and cavitation[208]. Furthermore, in various types of cells, such as for example Kupffer cells, hepatocytes, vascular smooth muscle cells, the increased generation of PGE₂ inhibited cytokine-stimulated NOS type 2 mRNA expression and NO synthesis in a concentration-dependent manner[211]. The effect of the prostaglandin was also associated with the number of macrophages[216]. All these disturbances could therefore affect innate and acquired immune responses of the host directed against oral prenatal or postnatal T. gondii infection, and result in generation of foamy macrophages and development of foamy colitis[213].

Frequent occurrence of a cryptogenic hypertransaminasemia in celiac patients (celiac hepatitis) reverting to normal after a few months of gluten withdrawal, as well as the presence of celiac disease in patients with primary biliary cirrhosis, autoimmune hepatitis, and primary sclerosing cholangitis[217], are in agreement with the above-presented reasoning[213]. Beneficial effects of diet with no gluten in patients with celiac disease may be explained by the fact that gluten induces a profuse intestinal cytokine response markedly predominated by IFN-γ (about 1000-fold in untreated disease)[220,219], and simultaneously there is also a significantly increased production of IFN-γ and other proinflammatory cytokines representing the host defense against concomitant latent T. gondii infection[220,219]. This overlapping excess of generation of interleukins in specific tissues of the host may finally be very destructive.

It must be emphasized that the presence of anti-tissue transaminase antibodies in patients with abnormal liver tests may not always solely suggest celiac disease[220,221]. Such comorbidities may be potentially harmful/beneficial for both humans and experimental animals because infecting pathogens can modulate inflammatory responses and final clinical outcomes[213,222,221]. Moreover, certain medicaments, such as for example disodium cromoglycate used in the patients with allergy, decrease activation of mast cells, and therefore attenuate parasite burden and decrease tissue lesions because its antihistaminic properties affect T<sub>1</sub>/T<sub>2</sub> cytokine balance in the host[222]. Moreover, in animals infected with RH strain of the parasite this drug decreased T. gondii tachyzoite surface antigen 1 gene expression in spleen and liver tissues[223].

In summary, literature data suggest that latent chronic T. gondii infection plays an important role in triggering and maintaining disturbances of so far unexplained liver function and histopathologic abnormalities observed in many clinical entities and healthy subjects. It seems therefore that individuals with clinical symptoms of liver disease and associated laboratory irregularities that are not helpful in establishing proper diagnosis, should have tests for the parasite routinely performed and receive specific pharmacologic treatment. Hypocholesterolemic properties of citrus flavonoids, especially naringenin and hesperitin, may help in reducing activity and expression of ACAT1, ACAT2 and microsomal triglyceride transfer proteins, thus limiting harmful effects of triglyceride accumulation in the endoplasmic reticulum lumen of the host cells[220-229]. In addition, hepcidin protease inhibitors that block T. gondii microneme protein secretion, glibitin motility, and host cell invasion may represent potent anti-parasitic agents capable of curing infections in vivo, including the chick embryo model of toxoplasmosis[220-222]. Moreover, the parasite cathepsin B and L proteins may represent potential novel DNA vaccine antigens against toxoplasmosis[221], especially that cathepsin B has recently been identified as β-secretase for the production of β-amyloid peptides that accumulate in Alzheimer’s disease[224] (this is in line with the above-mentioned suggestion that similar biochemical processes participated in the generation of amyloid present in MDBs). Interestingly, phenytoin (an antiepileptic drug) and cyclosporine A (an immunosuppressant) suppress the expression and activity of cathepsin L and B in cultured gingival fibroblasts[223,224]. Moreover, these two drugs stimulated TGF-β and cathepsin B production and inhibited expression of matrix metalloproteinase-1 by fibroblasts[223,227]. These are very important findings because they represent a novel mechanism of action of these two medicaments, and the above-presented reasoning is supported by the fact that chronic T. gondii infection was suggested to be the etiologic agent in cryptogenic episodes[228,229]. Recently, two groups[240,261] published clinical data supporting my suggestions that toxoplasmosis is a critical risk factor responsible for development of liver cirrhosis. In addition, an important role of T. gondii in the generation of nonalcoholic fatty liver disease has been suggested in a population-based studies[247]. The increasing prevalence of unexplained liver abnormalities[221], and overweight and obesity growing dramatically all over the world[248], are well in line with wide geographical distribution of the parasite. High burden of liver diseases and low awareness of toxoplasmosis amongst healthcare providers reported even in the United States require more effective public health actions[245,248].
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