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Direct evidence of *Toxoplasma*-induced changes in serum testosterone in mice

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ABSTRACT

Latent toxoplasmosis is known to influence the morphology of infected persons and also increases the probability of the birth of male offspring in both humans and mice. All these traits can be related to the observed differences in the concentration of testosterone between *Toxoplasma*-infected and *Toxoplasma*-free subjects. However, it is not possible to decide, using the *Toxoplasma*-human model, whether toxoplasmosis influences the level of testosterone in the infected host or whether individuals with different levels of testosterone vary in the probability of toxoplasma infection. Here we studied changes in the testosterone levels in the latent phase of toxoplasmosis in laboratory mice artificially infected with cystogenic but relatively virulent strain T38 of *T. gondii*. We observed decreased testosterone levels in both female and male mice with latent toxoplasmosis in comparison to uninfected controls ($P = 0.001$). The present results indicate that *Toxoplasma* infection changes the concentration of serum testosterone in mice and human rather than changed concentration of testosterone influences the probability of the *Toxoplasma* infection. It is possible that the decrease of testosterone is an adaptive mechanism of infected mice aimed to compensate toxoplasmosis-induced immunosuppression observed during latent *Toxoplasma* infection.

1. Introduction

Toxoplasma gondii (Apicomplexa) is one of the most common parasitic protozoans in humans. The prevalence of *Toxoplasma* infection varies mostly from 20% to 80% in different territories (Tenter et al., 2000). Latent toxoplasmosis is clinically asymptomatic, but usually life-long infection, characterized by the presence of *Toxoplasma* cysts, typically in the nervous and muscular tissues, and by life-long protective (both humoral and cellular) immunity to reinfection by the presence of low levels of anti-*Toxoplasma* IgG in the serum of infected individuals.

Latent toxoplasmosis is known to induce behavioral and neurophysiological changes in infected human or animal hosts (Webster, 2001), and is known to influence the morphology of infected persons. Infected men are higher, have a lower second to fourth digit length ratio in the left hand (Flegr et al., 2005, 2008a), i.e. have longer fingers (Kratochvíl and Flegr, 2009), and their faces in photographs are rated by female raters as more dominant and masculine (Hodková et al., 2007). All these traits can be related to the higher concentration of testosterone observed in *T. gondii*-infected men (Flegr et al., 2008b). Latent toxoplasmosis is also known to increase the probability of the birth of male offspring in both humans (Kaňková et al., 2007a) and mice (Kaňková et al., 2007b). James (2010) hypothesizes that many parasites and pathogens change the concentration of steroid hormones, here testosterone and estrogen, of infected hosts which often results in a shift in the sex ratio, namely in the increase of the proportion of males in the offspring. Whether toxoplasmosis induces the observed hormone concentration shift or whether subjects with changed hormone levels have a different probability of *Toxoplasma* infection cannot be told. For obvious reasons, the causality of the association between toxoplasmosis and hormone shift cannot be studied in a human model with artificial infection. The main purpose of the present study is to determine what is the cause and what is the effect using an animal model of infection. More precisely, we studied changes in testosterone concentration in laboratory mice artificially infected with a cystogenic strain of *T. gondii*.

2. Material and methods

2.1. Experimental animals and infection

In the experiment, a total of 81 mice, 41 females and 40 males of the F1 generation, cross-breeds of BALB/c female mice and C57 Black male mice, were used. At the age of 5–6 weeks, approximately half of the female mice ($N = 20$) and half of the male mice ($N = 20$) were orally infected with brain homogenate from mice infected with cystogenic but relatively virulent strain T38 of *T. gondii* isolated on April 18, 1975 by Zástěra from oocysts released by a

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stray cat originated from Southern Slovakia (Kodym et al., 2002). Homogenate was placed on a microscope slide, mounted with a coverslip and the number of *Toxoplasma* cysts in 0.2 ml aliquot was counted using binocular microscope at 100× magnification. Each mouse of the "infected" group was given orally approximately ten tissue cysts. The controls were given the same amount of isotonic saline (0.8% NaCl). The mice were maintained in groups of five per cage. The testosterone levels were analyzed in the serum. Blood samples were collected at 2 months after the acute infection (Kodym et al., 2002). Blood specimens (0.5–1 ml) of were obtained from the tail vein of both infected and control mice. Approximately 5 min elapsed between taking animals from cage to finishing blood collection. The efficiency of the experimental *Toxoplasma* infection in mice was confirmed by the complement fixation test (CFT) for the detection of specific anti-*Toxoplasma* antibodies IgM and IgG (namely the complement-binding subclasses IgG2a and IgG2b) (Ondriska et al., 2003). Infected mice without detectable levels of anti-*Toxoplasma* antibodies (seven female and eight male mice) and one infected female for which a sufficient amount of serum was not available were excluded from the analysis.

2.2. Testosterone determination

Testosterone assays were performed at the Institute of Endocrinology, Prague. 120 µl sera diluted with 380 µl of 0.85% sodium chloride were double-extracted with diethyl ether (2 × 2 ml) in stoppered glass tubes (vortex, 1 min). The aqueous phase was frozen in solid carbon dioxide, the organic phase (ether) was decanted to other pure analysis glass tubes and the ether was evaporated to dryness. The extracts were dissolved in 100 µl working buffer. A standard curve consisting of 0, 0.314, 0.627, 1.25, 2.5, 5.0, 10.0, 20.0, and 40.0 nmol/l testosterone (Koch Light Laboratories, England, UK) in duplicate was prepared. Antiserum (rabbit anti-testosterone-3-CMO: BSA, working dilution 1:80 000) and the tracer ([^{125}I] iodohistaminyl testosterone derivative, 15,000 cpm 1,863,000 cpm/10 µl), 100 µl each (Hampl, 1994), were added, the volume was adjusted to 100 µl with the working buffer (20 mmol sodium phosphate saline containing sodium azide and BSA, 0.1% each) and the tubes were equilibrated at 37 °C for 30 min and 1 h at 4 °C or overnight at 4 °C. After incubation 0.5 ml of dextran-coated charcoal suspension (2.5 g/l of Norit A and 0.25 g/l of dextran), was added to each tube to separate the free fraction and the radioactivity of ^{125}I was measured in the supernatant using a 12 channel gamma counter (Berthold, FRG). Results were calculated from the standard curve using a log–logit transformation, corrected for recovery and expressed as nmol of testosterone per liter of sample.

2.3. Statistics

The data were statistically analyzed using Statistica® 8.0 software. Since the levels of testosterone were not normally distributed even after log transformation, nonparametric tests, i.e. the Mann–Whitney test with a binary variable toxoplasmosis (positive/negative) and a continuous variable level of testosterone, and partial Kendall correlation with binary variables toxoplasmosis and sex (the probable confounding variable) and a continuous variable level of testosterone, were used to assess the influence of toxoplasmosis on serum testosterone levels (Siegel and Castellan, 1988; Sheskin, 2003). The Excel sheet for the computation of the partial Kendall correlation for one confounding (ordinal or continuous) variable is available at http://web.natur.cuni.cz/~flegr/programy.php.

3. Results

The final statistical analysis included 65 mice, 21 control and 12 infected female mice, and 20 control and 12 infected male mice. The nonparametric correlation tests showed that latent toxoplasmosis strongly affects the levels of testosterone in infected mice. The results of the partial Kendall correlation with sex of mice as a covariate showed that infected mice had significantly lower concentration of testosterone than controls (Tau = −0.271, $P = 0.001$). The effect of toxoplasmosis on testosterone levels was also analyzed separately in females and males. The analyses showed that both female and male mice with latent toxoplasmosis had significantly lower levels of testosterone (females: $Z = -2.32$, $P = 0.020$; males: $Z = -2.76$, $P = 0.005$), see Fig. 1.

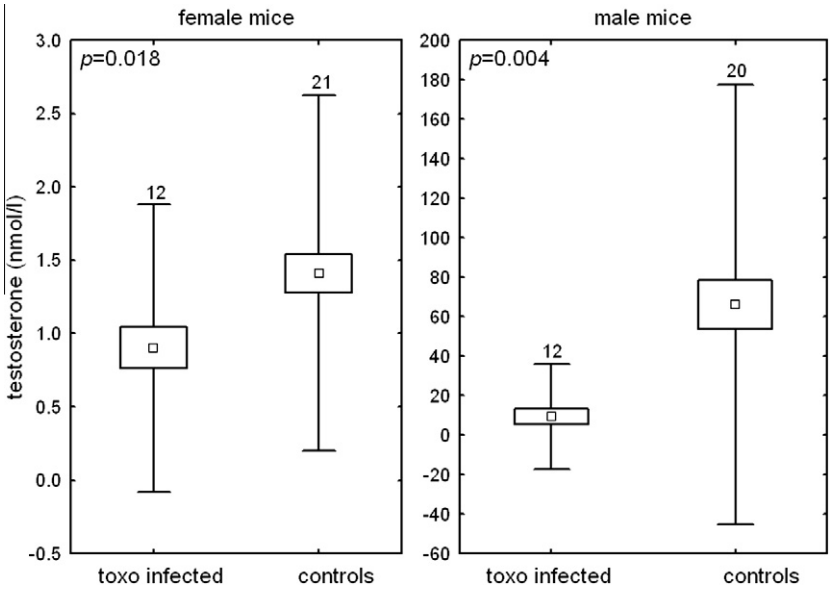

Fig. 1. Differences in serum testosterone levels between *Toxoplasma*-infected and control female and male mice. The boxes and spreads show the mean, standard error and standard deviation. The numbers under the boxes show the number of mice in each category.

4. Discussion

Toxoplasma-infected mice, both females and males, had lower serum testosterone concentration than controls. Previous studies (Flegr et al., 2005, 2008a; Hodková et al., 2007) could not determine whether *Toxoplasma* infection induces changes in testosterone concentration or whether low- and high-testosterone subjects differ in the probability of acquiring *Toxoplasma* infection. Our results clearly showed that the former assumption is correct, more precisely that toxoplasmosis influences the level of testosterone.

High concentrations of testosterone are known to have immunosuppressive effects (Roberts et al., 2001; Schuster and Schaub, 2001). Therefore, the results of the present study, namely the decreased and not increased concentration of testosterone in *Toxoplasma*-infected mice, make the immunosuppression-based explanation of the association between *Toxoplasma* infection and testosterone concentration rather unlikely. On the other hand, it could be speculated that the decrease of testosterone concentration could be an adaptive response of infected mice to toxoplasma-induced immunosuppression. By decreasing the concentration of testosterone, the infected mice could partly compensate the latent toxoplasmosis-associated down-regulated cellular immunity, namely the observed suppressed reactivity of macrophages and lymphocytes to the antigen in in vitro assays (Kaňková et al., 2010). Such compensation might increase the probability of the survival of infected mice after contact with various pathogens in their natural environment.

The results of our study do not agree with the predictions of James' hypothesis (James 2010) suggesting that the increased proportion of males in the offspring of *Toxoplasma* infected women and female mice is a direct effect of toxoplasma-induced increase of testosterone in infected hosts. He provides indirect evidence that this is the case during the infection with hepatitis B and C viruses and direct evidence that the shift occurs during latent infection with *T. gondii*. An alternative hypothesis explaining the *Toxoplasma*-associated sex ratio shift suggests that the phenomenon is caused by a higher probability of survival of more immunogenic male embryos due to *T. gondii*-induced immunosuppression (Kaňková et al., 2010). Actually, both hypotheses may be compatible as the proximate mechanism of immunosuppression remains unknown and might involve the parasite-induced shift in steroid hormones.

Moreover, the observed decrease of testosterone concentration contrasts with the observed increased testosterone level in infected male students. It is possible that the men with increased levels of testosterone have a greater chance of being infected by toxoplasmosis, either due to impaired immunity (Kaňková et al., 2010) or due to changed behavior (Lindová et al., 2006, 2010) and personality profile, e.g., their tendency to disregard rules of their society which can result in lower hygienic standards (Flegr, 2010) and, correspondingly, increased risk of contact with a source of infection. It is also possible that the physiological reaction to *Toxoplasma* infection differs qualitatively between mice and humans. The mouse is a typical short-lived species in which the duration of acute infection is comparable with the length of life. We used a cystogenic strain of *Toxoplasma* with moderate virulence and a low infection dose in our experiment and we measured the concentration of testosterone 8 weeks after the infection when all symptoms of acute toxoplasmosis had disappeared and even the weights of infected mice had returned to normal. However, toxoplasmosis is a rather serious disease in mice, in contrast to immunocompetent humans, and it cannot be ruled out that the effects observed in various mice studies are the carried over effects of acute toxoplasmosis rather than direct effects of latent toxoplasmosis (Hrdá et al., 2000; Kannan et al., 2010).

In the future, more results supporting the observation of increased testosterone concentration in men should be collected for various populations, to see whether the increase of testosterone is a general phenomenon in men or is specific only for male students. Moreover, the infection experiments should be repeated with other animal species, preferably long-lived ones, e.g., with *Toxoplasma*-resistant animals such as cattle or sheep, that are much better models for human latent toxoplasmosis than rodents.

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