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Original article

# An opposite role is exerted by the acarian *Myocoptes musculinus* in the outcome of *Toxoplasma gondii* infection according to the route of the protozoa inoculation

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**Abstract**

Infection with *Toxoplasma gondii* leads to a Th1 immune response. Alternatively, the acarian *Myocoptes musculinus* induces a disease in BALB/c mice that involves Th2 immune mechanisms. In this study, we investigated whether infestation by *M. musculinus* induces Th2 immune response in C57BL/6 mice and if this response influences the *T. gondii*-induced Th1 response when mice are inoculated by intraperitoneal or oral route. The animals were infected with *M. musculinus* and one month later with *T. gondii* ME-49 strain and the survival and immune response were monitored. The co-infected animals displayed higher mortality rate and the spleen cells showed a decreased IFN-γ and elevated IL-4 and IL-5 production. These changes were associated with severe pneumonia and wasting condition. On the other hand, when mice were orally infected with 100 *T. gondii* cysts, co-infection prolonged the survival rates and ameliorated intestinal lesions in association with a significant drop in IFN-γ levels in sera. These results indicate the interference of Th2 response induced by *M. musculinus* in a *T. gondii*-induced Th1 response. Altogether, these data demonstrate the profound interactions between the immune response induced against unrelated organisms *T. gondii* and *M. musculinus*, and suggest that this type of interactions may impact clinical disease.


*Keywords:* *T. gondii*; *M. musculinus*; Th1/Th2 response; Co-infection; Intestinal inflammation

## 1. Introduction

*Toxoplasma gondii* infection in most adult animals and humans is asymptomatic because of effective protective immunity [1]. In immunocompetent individuals, infection with the parasite causes little or no overt signs of disease in its hosts, but in situations of immunodeficiency, or during congenital infection, *T. gondii* may emerge as a serious infection, which if not treated can lead to host death [2,3]. Different studies performed with mice show the important role of cytokines, such as IL-12, TNF-α, and IFN-γ, and generation of reactive nitrogen intermediates (RNI) as mediators of host resistance to early *T. gondii* infection [3]. Resistance in both

*Abbreviations:* CNS, Central Nervous System; STAg, Tachyzoite Soluble Antigen; EI, ELISA Index; p.i., post-infection.

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acute and chronic infection with *T. gondii* in the murine model is highly dependent on endogenous IFN-γ [3–7]. However, if not controlled, the immunopathological mechanisms induced by the parasite can be detrimental to the host. C57BL/6 mice orally infected with 100 cysts of *T. gondii* develop an exacerbated and fatal intestinal inflammatory response, associated with the production of high levels of IFN-γ, tumor necrosis factor (TNF), and RNI [8,9].

Scabies is an important endemic disease of humans and other mammalians throughout the world. It is caused by the mite *Sarcoptes scabiei* that burrows in the stratum corneum of the skin. Worldwide, at least 300 million persons are estimated to be infested with scabies [10] and it is an important health problem in many developing countries [11]. *Myocoptes musculinus* is an acarian parasite that is the etiological agent of a disease in BABL/c mice, which is related to Mite-Associated Ulcerative Dermatitis (MAUD) described in the C57BL/6 mice and caused by *Myobia musculi*, a fur mite closely related to *M. musculinus* [12]. Like *S. scabiei*, both of them are members of the class Arachnida, however these parasites are purely surface dwellers and do not penetrate the deeper layers of the skin at any stage of their life [13]. In BALB/c mice, *M. musculinus* leads to a significant immunological disorder resulting in a T-helper-2 (Th2) type response, with allergic mechanisms, which are characterized by erythematous and pruritic skin lesions and increase in the specific-IgE levels [13,14]. This condition is similar to scabies in humans and other animals; for example, in human high serum levels of total IgE and IgG and eosinophilia are observed in addition to an elevated IL-4 production [15,16].

(retirei uma frase) Since co-infections are a widespread problem, analysis of the immune response in mice with concurrent infections has gained increasing interest. Particular attention has centered on the analysis of immune responses to concurrent infections with Th2- and Th1-inducing organisms, since these responses may have reciprocal counterregulatory properties. It was demonstrated recently that mice presenting concurrently infected with *Schistosoma mansoni* and *T. gondii* undergo accelerated mortality, which is preceded by severe liver damage [17,18]. In contrast, it was showed that *Nippostrongylus brasiliensis*-induced Th2 responses fail to alter *T. gondii*-induced Th1 responses and immunopathology [19].

We became interested to determine how the immune system responds when the host is co-infected with two contrasting parasites, as *T. gondii* and *M. musculinus*. In addition, we were also interested in the outcome of *T. gondii* infection under the influence of Th2 response, when the host are infected by different routes, intraperitoneal where the Th1 response are critical to avoid mortality and oral where the Th1 exacerbated immune response leads to mortality. We observed that the acarian infestation in mice accelerated the mortality rates when they were intraperitoneally infected with 10 cysts of *T. gondii*, suggesting that the acarian parasite disturbed the Th1-protective immune response to *T. gondii*. In contrast, when animals were orally inoculated with 100 cysts of the parasite, the acarian infestation decreases the intensity of the intestinal lesions by *T. gondii* and delays the mortality rates. These results suggest that on one hand the immune response induced by the mange does interfere in the type-1 immune response induced by *T. gondii* and it is detrimental to the host, but, on the other hand, in some condition this response can control the severity of the intestinal lesions induced by an excessive Th-1 immune response.

## 2. Materials and methods

### 2.1. Animals

The C57BL/6 mice were bred as homozygotes and kept in the Bioterism Centre of the Animal Experimentation Laboratory, Biomedical Sciences Institute, from our University. Mice were housed under specific pathogen-free conditions. All animals used for the experiments were females at age of 8 to 12 weeks. All procedures were conducted according to institutional guidelines for animal ethics.

### 2.2. M. musculinus and T. gondii infection

*M. musculinus* was maintained by keeping Swiss mice infested by this ectoparasite in isolated clean room with air filtration. The low-virulent ME-49 strain of *T. gondii* was maintained by intraperitoneal inoculation of C57BL/6 mice with brain homogenate. The infestations by *M. musculinus* were made by keeping together Swiss infested with C57BL/6 mice. The infestation was monitored by periodically taking skin scrapes that were examined under a microscope and by clinical examination of the animals. One month later, the *M. musculinus*-infested mice were infected with *T. gondii* by inoculation of brain homogenate containing 10 or 100 cysts by intraperitoneal or oral route, respectively.

### 2.3. Experimental procedure and tissue processing

C57BL/6 mice *M. musculinus* infested one month earlier were infected with 10 *T. gondii* cysts and groups of 3 mice were killed by cervical dislocation at 7, 14, 21 and 30 days post-infection (p.i.). Blood samples were collected for serological assays and tissue samples, such as Central Nervous System (CNS), lung, liver, and spleen were collected, fixed in 10% buffered formalin and processed routinely for paraffin embedding and sectioning. In other groups of experiments, *M. musculinus*-infested mice were orally infected with 100 *T. gondii* cysts by gavage. The small intestine was cut into four pieces and rolled on itself in order to make a ''Swiss roll''. Tissue sections with 4 μm thickness (40 μm distance between sections) of each organ from each mouse were obtained in microtome and mounted in slides for histopathological and immunocytochemical assays.

### 2.4. Histological analysis

In order to verify the histopathological changes, tissue sections were stained with Hematoxylin and Eosin or Toluidine blue and examined under light microscope. The inflammatory