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# Contrasting effects of *Trichinella spiralis* and *Trichuris muris* antigens on the infection by *Leishmania infantum* in BALB/c mice

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**Abstract**

The interaction of *Trichinella spiralis* and *Trichuris muris* derived antigens with the infection by *Leishmania infantum* was investigated in BALB/c mice. Infection with $10^6$ promastigotes of *L. infantum* did not induce relevant serum antibody (IgG subclasses), nor cytokine (IFN-γ, IL-4) responses despite that mice could partially control the infection. Immunization with *T. spiralis* activated a moderate IgG1 and secondarily an IgG2a anti-leishmanial response whereas immunization with *T. muris* elicited only a weak and late activation of IgG1 anti-leishmanial response. Immunization with *T. muris* caused an elevation of serum IFN-γ levels which was drastically reinforced by the *L. infantum* infection, and that was accompanied by almost complete parasitological cure of infected mice. Immunization with *T. spiralis* induced an elevation of serum IL-4 levels but this response was greatly (about 60%) neutralized by the infection with *L. infantum*, and this was associated to exacerbation of the infection.


*Keywords:* *Leishmania infantum* infection; *Trichinella spiralis*; *Trichuris muris*; Antigens; IgG subclasses; IFN-γ; IL-4

## 1. Introduction

Among experimental models used for visceral leishmaniasis caused by *Leishmania donovani* and *L. infantum*, the BALB/c mouse strain has been one of the most extensively employed. Although initially susceptible to experimental infection, usually achieved under administration of highly infective doses ($10^8$ parasites) of virulent strains, BALB/c mice became able to control the extension of the infection with parasite burden being reduced higher than 85%, mainly in liver (Leclercq et al., 1996; Mukherjee et al., 2003) with acquired resistance to challenge (Murray et al., 1992). This resistance is based on activation of Th1 type response through the production of IFN-γ and IL-2, thus promoting macrophage activation and inflammatory granulomatous reactions (Murray et al., 1987). Upon macrophage activation inducible nitric oxide synthase (iNOS) is triggered for the production of nitric oxide (NO) as the major weapon for killing intracellular amastigotes (Liew et al., 1990; Colasanti et al., 2002). However, the same strain of mice can mount a predominant Th2 type response when infected with *L major* and consequently failing to control the cutaneous infection (Carter et al., 1989; Chatelain et al., 1992; Leal et al., 1993). Moreover, concomitant activation of Th1/Th2 responses was seen in animals infected with *L. infantum* (Rolao et al., 2007). This dual

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behaviour indicates that against *Leishmania* infection in BALB/c mice both Th1/Th2 responses can be activated and the resultant resistant or susceptible phenotype is merely dependent on the balance of these 2 antagonist responses respectively (Heinzel et al., 1989; Miralles et al., 1994).

Alternatively, the control of infections by gastrointestinal helminths is mainly dependent of the activation of Th2 response throughout the production of IL-4, IL-5, IL-10 and IL-13 that promotes increased eosinophilia, mastocytosis and IgE responses (Urban et al., 1996; Finkelman et al., 1997). Mouse models of study of helminth infections are largely represented by the trichuroid nematodes *Trichinella* and *Trichuris*. Upon infection by *Trichinella* species an initial Th1 response is activated during the first stages of the intestinal phase which eventually turns to a selective Th2 phenotype (Ishikawa et al., 1998; Helmby and Grencis, 2003). This response, in combination with innate immune events, lead to an acute inflammation that causes expulsion of the established intestinal adult worm population (Knight et al., 2004). Meanwhile the new born larvae released by females in the intestine progress to its settlement in muscles where they elicit a delayed type hypersensitivity reaction thus leading to a chronic inflammation stage (Li and Ko, 2001). Mice experimentally infected with the mouse whipworm *T. muris* selectively promote Th1 or Th2 responses during the first weeks of intestinal infection according to the genetic background of these mouse strains (Else and Wakelin, 1988; Else et al., 1990). Those predominantly expressing Th2 phenotype will be able to expel the worms by the 3rd week of infection and will become resistant to re-infections whereas those that mount a predominant Th1 response will become susceptible to infection allowing its progress to the chronic stage (Else et al., 1989; Else and Grencis, 1991; Else and de Schoolmeester, 2003). In this manner *T. muris* represents the paradigm of immune responses against gastrointestinal helminth parasites (Cliffe and Grencis, 2004).

Based on this background information we could expect that the concurrence of these nematodes would help to increase the susceptibility of the BALB/c mouse strain to *L. infantum* infection. Besides, these three parasites could constitute useful models to study immunological interactions of helminths with protozoa and their mutual consequences in concurrent infections. To approach these goals in the present paper we sought to assess how the presence of *T. spiralis* or *T. muris* antigens effect the course of an infection by the protozoa flagellate *L. infantum* in the resistant BALB/c mice and the immunological implications underlaying this effect.

## 2. Material and methods

### 2.1. Parasites

#### 2.1.1. T. spiralis

The isolate of *T. spiralis* used was MFEL/SP/62/GM-1. Parasites were maintained in CD1 mice and first stage (L1) muscle larvae were obtained after artificial digestion of carcasses from infected mice using the method of Wakelin and Lloyd (1976). After selection of live larvae by the Baermann method, they were washed ten times by sedimentation in PBS pH 7.2–7.4.

#### 2.1.2. T. muris

The strain of *T. muris* (Edinburgh strain) was originally isolated from *Mus musculus* in the Zoologic Park of Edinburgh and kept in the Wellcome Research Laboratories until it was donated to Professor Wakelin (Glasgow). Since 1988 it has been maintained in our laboratory by periodical passages in Swiss CD-1 mice.

#### 2.1.3. L. infantum

The authoctonous isolate M/CAN/ES/96/BCN150 (Zymodme MON-1) of *L. infantum* was kindly provided by Drs. Alonso and Requena in the year 2000. Since then it was maintained in our laboratory by periodical passage in Syrian golden hamster.

### 2.2. Mice

BALB/c mice of 6–8 weeks of age were purchased from Harlan Ibérica S.A. (Barcelona, Spain) and allocated in the Animal House Unit of the Complutense University under controlled food, light/darkness cycles and temperature conditions. All procedures of animal manipulations were approved by the Complutense University Institutional Animal Care and Use Committee following Spanish law.

### 2.3. Antigens

Antigen preparations from nematodes were chosen according to those commonly used in mouse vaccination assays.

#### 2.3.1. Preparation of Trichinella larval crude extracts (LCE)

After being washed, the settled larvae obtained by artificial digestion and selection by Baermann were sonicated in a Virsonic 5 sonicator (Virtis, NY, USA) for several 10 s pulses at 70% power on ice bath. The crude extract was extracted in PBS buffer pH