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Physiological responses to *Trichinella spiralis* infection in Wistar rats: Is immune response costly?

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Summary

Several studies try to measure immunological costs by the injection of non-pathogenic substances eliciting immune responses. However, as infectious agents are causing damage to hosts, energy consumed by hosts exclusively due to immune response is difficult to measure. In this study, we use a host/parasite model (rat/*Trichinella spiralis*) which allows to know the cost of immune response against a pathogen as compared with that to inert antigens. Tissue damage caused by *T. spiralis* during reinfection is scant if any, thus allowing the comparison of costs of secondary immune response to erythrocytes and immunity to reinfection by *Trichinella*. We compared the mass-controlled oxygen consumption in response to infection with *T. spiralis* and to injection with non-pathogenic heterologous erythrocytes in Wistar rats (primary and secondary responses). The results showed that mass-controlled oxygen consumption did not increase significantly in rats injected with erythrocytes during primary or secondary response. However, mass-controlled oxygen consumption in *T. spiralis* infected rats has showed: (i) a clear tendency to increase during primary response, specially in acute phase of infection and (ii) a significant increase during earlier secondary response as compared to rats injected with erythrocytes and control rats. We concluded that injection with this type of substances may be relatively uncostly as compared to those elicited by pathogenic agents.

Key words: *Trichinella spiralis*; SRBC; immune response; oxygen consumption

Introduction

Fitness costs of parasitism have been shown in several studies, especially in terms of reproductive success, survival and fecundity (see review by Møller, 1997). Costs of chronic infections are usually more difficult to detect, but subtle although important effects may occur in most cases (Munger and Karasov, 1989). Metabolic changes due to parasitism have been reported in several species (Kilgore *et al.*, 1988; Booth *et al.*, 1993). Together with direct cost produced by parasites (i.e.: withdrawal of resources, tissue damage), hosts must allocate resources to reduce parasite effects and invasion. However, the real costs for the organism of mounting an effective immune response are unknown and difficult to calculate (Hillgarth and Wingfield, 1997), as direct and indirect costs are so intimately associated that they may be difficult to separate.

The aim of this study is to measure energetic cost (by means of oxygen consumption) caused directly by immune response in Wistar rats infected with *T. spiralis* as compared to that caused by the injection of an inert antigen (SRBC: sheep red blood cells). In addition, physiological changes in mass, temperature and food intake were also checked. We select the injection with SRBC due to their common use in several recent works measuring physiological costs (Williams *et al.*, 1999; Cichon, 2000). The Wistar rat-*T. spiralis* system is specially appropriate for this comparison as *T. spiralis* is a parasite widely studied and immune responses against *Trichinella* infection in rats are well known (Bell, 1998). During reinfection *Trichinella* larvae are rejected at intestinal level mostly based on immune responses (Appleton and McGregor, 1984; Bell, 1998) thus allowing the study of physiological costs due exclusively to immunity.

Material and Methods

Experimental treatment

Fifteen male Wistar rats (4 months old) were used for the experiment. Animals were maintained in individual cages and all environmental conditions followed European guidelines 86/609/CEE and 93/119/CE.

Rats were randomly assigned to one of three treatments; 1) SRBC or S-rats: Five rats were injected intraperitoneally (i.p.) with 1 ml of sheep red blood cells (10 % suspension; Sigma) in non-pyrogenic isotonic solution (Pharmacia). 2) *Trichinella* or T-rats: Five rats were inoculated orally with 4000 L_1 (muscle) larvae of *T. spiralis* (GM-1 strain) in 1 ml of physiological saline. Larvae were harvested after artificial digestion of mice infected carcases and immediately inoculated to rats. Animals were infected with a sub-lethal dose of larvae known to produce a chronic infection and a high humoral response in Wistar rats (Martinez *et al.*, 2001). 3) Control or C-rats: two rats were injected (i.p.) with one ml of non-pyrogenic isotonic solution and 3 rats were provided orally with 1 ml of physiological saline as a control for both treatments.

Oxygen consumption, body weights, rectal temperature and food intake were measured on days -1, 1, 7, 14, 20, 27, 34, 41, 56, 62, 69 (day 0 = day of inoculum of *T. spiralis* and SRBC injection). On day post-infection 72 d.p.i. rats from every group were treated again as on primary infection and oxygen consumption measured at 16 h, 40 h, 5 and 7 days post-re-infection (d.p.r.i.) to study the effect of reinfection and secondary response to SRBCs on oxygen consumption. Measures were taken at these times due to the fast immune reaction against reinfection (< 24 hours d.p.r.i.). Rats were sacrificed on 7 d.p.r.i. to obtain the number of larvae present in muscle and to evaluate the response to SRBC (see below). In order to control for infectivity of larvae, two mice were infected with larvae from the same pool used to reinfect experimental rats. Thirty days later both mice were sacrificed and found infected.

Oxygen consumption

Oxygen consumption was measured using an oxygen analyser (Binos 100 2M, precision 0.01 %; measurement protocol C of Hill 1972). Oxygen volume normally stabilised after 15 min, although we kept rats in the chamber for 25 min in order to obtain a sufficient safety margin. We recorded the percentage of oxygen during the last five min at intervals of one min (total five times). The difference between oxygen percentage in atmosphere and average of the five measures was used as an estimate of individual oxygen consumption (Senar *et al.*, 2000). That value was divided by the mass of every rat to calculate a mass-corrected oxygen consumption. The chamber of the respirometer was a plastic cylinder with a volume of 3050 ml (12 cm high and 18 cm of diameter) and air flow rate was 900 ml/min. All measurements for the 15 rats were obtained on the same day and at a constant temperature of 20ºC. Three hours before the oxygen consumption measure was taken, food was withdrawn in order to reduce the consumption changes due to digestion.

Mass change, temperature and food intake

Immediately after the measure of oxygen consumption was obtained, rats were weighed to the nearest gram and rectal temperature measured to the nearest 0.1ºC with a digital thermometer. Food was weighed and provided *ad libitum*. Food intake was calculated before every measure as the food consumed between measurements by weighing the food not consumed and subtracting it from the value of food provided.

Response to SRBC and number of larvae in muscle

ELISA was used to detect the presence of antibodies (IgGs) against SRBCs at the end of the experiment. ELISA plates were coated for 2 hours at 37°C with soluble SRBC antigens. Later, plates were blocked with defatted milk diluted in PBS buffer for 1 hour at 37°C. Serum from rats was added in serial dilutions beginning with 1/100 and incubated for 1 hour at 37°C. Following an incubation step with peroxidase conjugated anti-rat serum, immunoreactions were visualised by incubation with a substrate comprising ABTS and concentrate hydrogen peroxide diluted to 1/1000. Absorbances were quantified in a plate spectrophotometer at λ= 405 nm.

In order to check the success of infection procedures as well as the severity of infection produced, the number of larvae per gram of muscle was obtained for every infected rat at the end of the experiment.

Statistical tests

Oxygen consumption, food consumed and temperature were used as dependent variables in ANOVA in order to study their variation among experimental groups. Simple regression was used to correlate number of larvae/g with different variables. We use Isotonic regression (the statistic $\bar{E}^2$) instead of ANOVA to test hypothesis, because this technique is more powerful than analysis of variance when one wishes to test ordered predictions (Gaines and Rice, 1990). Due to the parasite effects on host we specifically expect: 1) higher cost during acute phase (approximately from day 7 to 20 post infection) for T-rats in terms of both metabolic costs and reduction in mass and food intake than controls and S-rats. The latter group may show a higher oxygen consumption than C-rats based in the cost produced by response to an inert antigen. 2) Once acute phase is surpassed and rats recover from mass loss the continuous stimulation of immune system by encysted larvae (Bell, 1998) should maintain higher oxygen consumption than for SRBC injected and control rats. 3) During re-infection, the immune response in the intestine rejects *Trichinella* larvae less than 24 hours post-infection (Appleton and McGregor, 1984; Bell, 1998), leading us to expect a higher oxygen consumption than for controls rats, with SRBC in an intermediate position.

Results

Before the beginning of the experiment there were no significant differences in mass, temperature, food consumed and oxygen consumption between rats assigned to different treatments ($P > 0.05$ in all cases).

Our ordered expectation in mass-controlled oxygen consumption *Trichinella*> SRBC>control between days 7 – 20

was not significant (P > 0.08). However, at 16 h.p.r.i. infected rats showed higher consumption than SRBC and control ones (T>S>C: $\bar{E}^2_3$=0.45, P=0.008). *Trichinella* rats showed a tendency to consume more oxygen than SRBC and control rats from 14 d.p.i. to the end of the experiment (Fig. 1A).

Between days 1 – 7 and 7 – 14 infected rats lost mass, but not control and SRBC rats ($\bar{E}^2_3$=0.79, P<0.001 and $\bar{E}^2_3$= 0.60, P=0.002, respectively, Fig. 1B). From day 14 to day 20 and from 20 to 27 infected rats began to recover and mass change was significantly higher than for control and SRBC rats ($\bar{E}^2_3$=0.79, P < 0.001 and $\bar{E}^2_3$=0.80, P < 0.001, Fig. 1B). During the rest of the experiment the three groups showed homogenous mass changes. Looking at the total mass, infected rats were significantly lighter than rats from the other groups from day 7 d.p.i. to 40 h.p.r.i. (P < 0.05 in all cases).

There were significant differences in the total food intake on days 7 and 14 with *Trichinella* infected rats consuming less food than rats from the other groups ($\bar{E}^2_3$=0.92, P=0.002 and $\bar{E}^2_3$=0.91, P=0.002, respectively; Fig. 1C). Rectal temperature did not show significant variation among groups for any experimental day (P > 0.05 in all cases).

The number of *Trichinella* larvae per gram of muscle recovered from infected rats averaged 796 ± 281 S.E. This number indicates the intensity of infection during the acute phase as have been found correlated with new born larvae produced (Wang, 1997). Thus, we correlate the number of larvae present in muscle with the variables under study. We found a positive relationship with mass-corrected oxygen consumption on days 7 and 14 (r=0.95, P=0.012 and r=0.94, P=0.018, respectively). A significant positive correlation also appeared between muscle larvae and rectal temperature on day 7 (r=0.96, P=0.008). At the end of the experiment the antibody titter against SRBC antigens was 400 in four rats and 3200 in one. As expected, only rats injected with SRBCs showed antibody response against SRBCs.

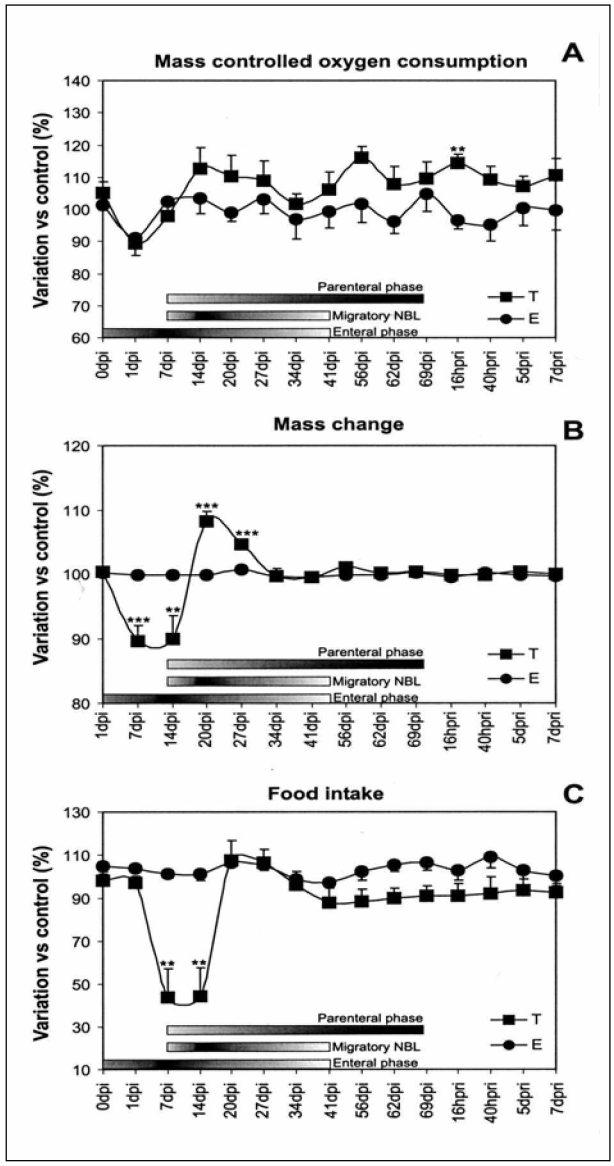

Fig. 1. Variation (%) of SRBC (E, circles) and *Trichinella* infected (T, squares) rats from controls (=100 %) for: A) Mass-corrected oxygen consumption, B) Mass change, C) Total food intake. Approximate duration and maximum intensity of different infection phases are shown (data from Despommier, 1983). dpi = days post infection, hpri = hours post reinfection, dpri = days post reinfection. Bars indicate SE. **P < 0.01 and ***P < 0.001

Discussion

Clear effects of *Trichinella* infection appear when comparing this group with controls and SRBC injected rats during primary response. As expected, mass loss is associated with infection by this parasite due to the inflammation and intestinal malabsorption produced during the enteral phase of the infection (Weatherly, 1983). Once the parasite abandons the intestine, rats begin to increase in mass at a higher rate than rats from the other groups in order to recover from malnutrition. Although the well-reported damage produced during acute phase (Campbell, 1983; Perez-Serrano *et al.*, 2000) could imply a higher mass-corrected oxygen consumption, from day 14 onwards infected rats only showed a tendency to increase this parameter (Fig. 1a). Nevertheless, the severity of infection influence the costs produced in hosts as shown by the significant positive correlation between mass-corrected oxygen consumption and number of larvae at 7 and 14 d.p.i. (acute phase), and temperature and number of larvae at 7 d.p.i. On the other hand, the injection of SRBC is not associated with any significant change in the variables measured, thus implying a low cost of response to this immune challenge.

Reinfection induced a significant increase in mass-corrected oxygen consumption at 16 h.p.r.i. as compared to reinjected and control rats (Fig. 1A). At this time reinfection is rejected at the intestinal level due to a strong immune reaction to *T. spiralis* (Appleton and McGregor, 1984; Bell, 1998). In fact, a stimulation of lymphocytes T at the intestine during the first infection is present in only 12

hours and even faster in reinfection responses (Bell 1998). Up to 99 % of worms are rejected by immune responses at the intestine level and cell damage, if any, is tremendously reduced (Bell, 1998). In the present study, the lack of differences in mass change and food intake between *T. spiralis* and control group during reinfection confirms the phenomenon of "rapid expulsion". In addition, in our previous studies with the same host-parasite model, stress protein levels at the small intestine increase during infection but not during reinfection thus pointing to a low cellular stress (Pérez-Serrano *et al.*, 2000). Therefore, the increase in mass-controlled oxygen consumption detected at 16 h.p.r.i. can be mainly claimed to immune response.

Although in laboratory conditions this immune response is a short-term and apparently not very high cost for rats the situation in the wild may be different. Several studies report trade-offs between investment in immunity and other functions based on challenges with inert antigens (Fair *et al.*, 1999). The present study shows that injection with SRBC is relatively uncostly at least for animals maintained in cage conditions (stable temperature and *ad libitum* food). Thus, responses to immune challenge with these substances may be short-term and energetically less expensive, being only detected under stressful circumstances. The presence of stress situations in the wild (cold spells, food scarcity, high growth requirements, etc.) may account for the evidence of costs to mount an immune response to the challenge, as well as for the short time involved in some of these trade-offs. The results of studies of trade-offs may be considered as conservative as responses to real infection should be higher.

In conclusion, at least secondary immune responses elicited by parasites may be more intense and metabolically costly than responses elicited by inert antigens. In the case of *Trichinella* the higher costs are produced soon after reinfection (16 h). Our results imply that trade-offs mediated by immunocompetence may be difficult to show in the wild as the intensity of the reaction against foreign substances may depend on the persistence and damage produced. However, costs due only to immunity against parasites may be of great importance in natural situations.

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