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Biphasic Hypothermia in Mice Infected with a Parasitic Nematode, *Trichinella spiralis*

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Abstract: In BALB/c mice infected with *Trichinella spiralis*, changes in body temperature (T_b) were observed over 35 days after the infection. *T. spiralis* infection induced hypothermia two times at 7 and 28 days after infection. The initial decrease persisted for about one week with a peak ($37.1 \pm 0.62^{\circ}\text{C}$) around 10 days after the infection, while the later phase persisted for at least one week. Both 10 and 35 days after the infection, there were remarkable decreases in T_b. The serum glucose level of infected mice at 10 days was significantly ($p < 0.01$) decreased compared with that of control mice at the same number of days, while the level in infected mice at 35 days was not decreased. Moreover, the later phase of hypothermia was prevented by the cyclooxygenase inhibitor indomethacin (10 mg/kg i.p.), while the initial phase was not. We conclude that hypothermia was caused by two different mechanisms, involving the effects of hypoglycemia and prostaglandins.

Key words: BALB/c mouse, Hypothermia, *Trichinella spiralis*

Several cytokines stimulate the synthesis of acute-phase proteins that play crucial roles in maintaining homeostasis during infection and inflammation [8]. Fever is one of the most widely recognized and clinically accepted features of the host defense response to infection and inflammation. Some cytokines such as interleukin-1 (IL-1) and IL-6 are the initial mediators of fever and recognized endogenous pyrogens. Moreover, prostaglandins are thought to be involved in the final stages of the pathogenesis of fever by actions on thermosensitive neurons in the hypothalamus. In concert with endogenous pyrogens, several other endogenous substances including a cytokine tumor ne-

crosis factor- α (TNF- α) and prostaglandin (PG)D₂ have been shown to exhibit antipyretic properties and are defined as endogenous cryogens [7, 14].

Trichinellosis is a serious zoonotic disease caused by a parasitic nematode *Trichinella spiralis*, and is the most common and well understood parasitic infection of skeletal muscle. Fever is known to be a major clinical symptom in human trichinellosis in the muscular phase. On the contrary, naturally infected animals do not show clinical signs [2, 4]. *T. spiralis* infection induces mild hypoglycemia in mice during the early period after the infection [13]. BALB/c mice have been well studied in experimental trichinellosis because they respond well

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with cytokine generation in *T. spiralis* infection [3]. In this paper, we describe a hypothermic response induced by hypoglycemia and prostaglandin effects in mice infected with *T. spiralis*.

Five-week-old male mice (BALB/cA; obtained from Clea Japan Inc. Tokyo) were used throughout our experiment. Mice were housed four per cage and allowed to acclimate for at least one week before the experiment under a standard 12:12 hr light-dark cycle in which the light period was from 7:00 a.m. to 7:00 p.m. Ambient temperature was kept at $24 \pm 0.5^\circ\text{C}$, and food and water were given *ad libitum*.

T. spiralis (ISS 412) was maintained by passages in mice (ICR; Clea Japan Inc. Tokyo) in our laboratory. For infection, infective muscle larvae (ML) were isolated from the mice by pepsin-hydrochloric acid digestion. Briefly, minced skeletal muscles were incubated for 2 hr at 37°C in digestion solution (5 g pepsin/l of 0.36% HCl), under constant stirring. After digestion, ML were allowed to sediment for 15 min at room temperature, and were washed three times in 0.9% NaCl. On average, 2×10^3 larvae/g of infected muscle were collected. Mice anesthetized with ethyl ether were infected with 100 ml/mouse orally with a probe. Uninfected mice were used as a control.

Tb was measured in the colon in mildly restrained (hand held) conscious mice and monitored every 2–3 days from 7 days before to 35 days after infection. Colonic temperatures were assessed by insertion, 2.5 cm beyond the rectum, of a thermocouple connected to an electrical thermometer (Thermalert TH-5, Physitemp Instruments INC., USA). Measurement started at 4:00 p.m. and finished around 4:30 p.m.

Mice were administered 10 mg/kg indomethacin (IND; Sigma, St. Louis, MO.) dissolved in 0.3% carboxymethyl-cellulose (CMC) or 0.3% CMC intraperitoneally at 10 and 35 days after infection. Administration of 0.3% CMC was designated as vehicle. Tb was measured 60 and 240 min after the injection by the method described above. Results were expressed as absolute change in the colonic temperature between post-injection and pre-injection values.

Serum samples were obtained from mice 10 and 35 days after infection with *T. spiralis*. Serum glucose was determined by an enzymatic glucose assay kit (Glucose CII-test Wako, Wako Pure Chem. Industrial Ltd. Tokyo).

Data are presented as means and SD ($n=5-16$). Statistical evaluation was carried out using one-way analysis of variance (ANOVA) with repeated measures and Student's *t* test to test for differences between the control and the infection groups. A *p* value of <0.05 was considered significant.

This study was approved by the Animal Research Committee of Saitama Medical School.

Before experimentation, normal mice exhibited a normal circadian rhythm in which their Tb was higher in early morning and lower in the late afternoon (data not shown).

Changes in Tb of the infected and control mice are shown in Fig. 1. *T. spiralis* infection induced hypothermia twice at 7 and 28 days after the infection. The initial phase decrease persisted for about one week with a trough ($37.1^\circ\text{C} \pm 0.62$) around 10 days after the infection, while the later phase persisted for at least one week. Tb of control mice did not change temperature during the experimental period.

Both 10 and 35 days after the infection, there were remarkable decreases in Tb (Fig. 1). The serum glucose levels in the infection and control groups were 126.8 ± 13.19 mg/dl ($n=16$) and 212.8 ± 40.00 mg/dl ($n=16$) at 10 days, and 193.2 ± 27.57 mg/dl ($n=16$) and 195.5 ± 18.09 mg/dl ($n=16$) at 35 days, respectively (Fig. 2). The glucose level in the infection group at 10 days was significantly ($p<0.01$) decreased compared with that in the control group at the same number of days, while the level in the infection group at 35 days was not decreased. These results are consistent with previous study by Stewart *et al.* [13], who demonstrated hypoglycemia in mice infected with *T. spiralis*. Hypoglycemia occurred in the host from the 3 to 20 days after *T. spiralis* infection with a trough around 10 days after the infection. Based on their results as well as our own, we conclude that hypoglycemia probably induced the initial phase of hypothermia. As a cause of hypoglycemia-induced hypothermia, Silvia and Boulant [10] demonstrated that a low-glucose tissue perfusion excites the majority of warm-sensitive neurons of the perioptic anterior hypothalamus, while it inhibits the central nervous system containing neural foci involved with the regulation of temperature.

The effects of IND on hypothermia induced by *T. spiralis* infection are shown in Fig. 3. Initial Tb of infection and control subjects were $37.4 \pm 0.58^\circ\text{C}$

Fig. 1. Changes in body temperature of mice infected with *T. spiralis*. Values are means $\pm$ SD. *: Significantly different from control at the same number of days.

Fig. 2. Concentration of serum glucose in mice 10 and 35 days after infection with *T. spiralis*. Open and filled bars are control and infected mice, respectively. Values are means $\pm$ SD. *: Significantly different from control at the same number of days.

($n=10$) and $38.3 \pm 0.26^\circ\text{C}$ ($n=10$) at 10 days, and $36.7 \pm 0.58^\circ\text{C}$ ($n=10$) and $38.7 \pm 0.59^\circ\text{C}$ ($n=10$) at 35 days, respectively. There were no differences in Tb between IND-treated [$38.2 \pm 0.04^\circ\text{C}$ ($n=5$)] and vehicle [$38.4 \pm 0.11^\circ\text{C}$ ($n=5$)] groups at 60 min after the injection, 10 days after the infection. By contrast, IND significantly ($p<0.05$) inhibited the decrease [IND: $38.4 \pm 0.23^\circ\text{C}$ ($n=5$); vehicle: $37.4 \pm 0.61^\circ\text{C}$ ($n=5$)] in Tb at 60 min

after the injections, 35 days after the infection. Tb of mice on both 10 and 35 days after the infection returned to almost the initial level 240 min after the injection treatment. In the twice recurring hypothermia in our result, IND treatment inhibited the later decrease, but not the initial decrease. Hence, the later decrease was associated with the mediation of prostaglandins (PGs), because IND is a cyclooxygenase (COX) inhibi-

Fig. 3. Effect of treatment with the cyclooxygenase inhibitor indomethacin (IND) on changes in body temperature of mice 10 and 35 days after infection with *T. spiralis*. Data are expressed as absolute change in body temperature between postinjection (60 and 240 min after intraperitoneal injection) and preinjection values. *: Significantly different from control mice injected with IND. CMC: carboxymethyl-cellulose.

tor which inhibits the production of PGs including PGD_2 . PGD_2 has been reported to exhibit antipyretic properties when injected into the brain [14].

The present study demonstrated that *T. spiralis* infection induced hypothermia two times in BALB/c mice. The most interesting new finding is that the causes of each hypothermia are not the same. Based on the results of IND treatments and serum glucose levels of the host 10 and 35 days after infection, we concluded that there are two different mechanisms that induce hypothermia after *Trichinella* infection.

PGs biosynthesis and hypoglycemia inducement are closely related to cytokine effects. In hypoglycemia inducement, infection or endotoxin administration to experimental animals produced several disturbances in carbohydrate metabolism associated with the cytokine effects [12]. Recent studies have shown that administration of $\text{TNF-}\alpha$ and IL-1 to mice produces a decrease in serum glucose levels [6]. In PGs biosynthesis, $\text{TNF-}\alpha$ and Interferon γ (IFN- γ) synergize to induce COX-2,

one of the isoenzymes of COX, mRNA expression in the macrophage [1].

Frydas *et al.* [5] observed in detail cytokine generation of BALB/c mice infected with *T. spiralis*. The mice showed increased $\text{TNF-}\alpha$ 15 days after infection, which reached a maximum at 35 days. IFN- γ was enhanced 20 days after infection and the maximal level of this cytokine was reached at 35 days. Regarding IL-1 production, its mRNA was observed in the host rat by Stadnyk [11] who detected markedly increased expression of IL-1 mRNA in several organs of the host 4 days after infection.

Based on the results of the present study using BALB/c mice and data existing in the literature, the involvement of cytokines ($\text{TNF-}\alpha$, IL-1 and IFN- γ) in *T. spiralis* infection-induced biphasic hypothermia is suggested. One of the possibilities involves the roles of IL-1 for the initial phase, and $\text{TNF-}\alpha$ and IFN- γ for the later phase as mediators of the hypothermia response. Although our results are based on hypothermia in BALB/c mice in-

fectured with *T. spiralis*, there is insufficient evidence to explain the effects of the cytokines. Moreover, fever is a major clinical symptom in human trichinellosis [2], which is different from our results. Fever is developed by the action of PGE₂, an endogenous pyrogen, induced by infection [7]. In the cascade of PGs generation, it is well known that PGH₂ is the precursor of both PGE₂ and PGD₂ production through PGE₂ and PGD₂ isomerases, respectively [8]. Thus the differences in thermoreactions between human and mouse infected with *T. spiralis* might be explained by the differences in the enzymic balances among PGH₂, PGE₂ and PGD₂. Further study is necessary to clarify the processes occurring during *Trichinella* infection in different host species.

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