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MALNUTRITION AND GLUCOCORTICOIDS THERAPY IMPAIR HOST IMMUNE RESPONSE DURING *TRICHINELLA SPIRALIS* INFECTION

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Abstract

Trichinosis is an important food-borne zoonotic disease worldwide, caused by a nematode *Trichinella spiralis*. Malnutrition and glucocorticoids (GC) therapy are the commonest causes to diminish human immune responses.

This study evaluated the effects of malnutrition as well as GC therapy on host immune response during *T. spiralis* infection.

One group of albino mice (mal-nourished) received a protein-deficient diet (6.5% casein), other three groups (control non infected, infected well-nourished and infected GC-treated mice) received a control diet (20% casein). GC-treated mice were given dexamethasone intraperitoneally at a dose of 25ug/day for three weeks before infection and continued to receive dexamethasone throughout the experimental time. Mice were orally infected with250 larvae/mouse and sacrificed on 7th & 21st day post infection (p.i.) and adults in intestine and larvae in diaphragm were examined. Cytokines and pro-inflammatory mediators 'sera were measured by ELISA.

The results showed that mean number of adult worms in intestine and larvae in diaphragm were significantly higher in mal-nourished and GC-treated than well-nourished mice. Infected mal-nourished and infected GC-treated mice, had lower serum concentration of Th1 (IFN-y & IL-12) and Th2 (IL-4 & IL-10) cytokines than corresponding well-nourished group. Similar results were obtained with pro-inflammatory mediators (TNF-α, IL-1β & IL-6) that were lower in infected mal-nourished and infected GC-treated than infected well-nourished mice.

Key words: *Tricinella spiralis*, malnutrition, glucocorticoids, immune response.

Introduction

Trichinella spiralis is a worldwide distributed zoonotic parasite and about 11 million trichinosis patients with a heavy infection suffered serious muscle pain and sever morbidity or even death (Gottstein *et al* 2009). In Egypt, *Trichinella* encysted larvae were reported in slaughtered pigs (Morsy *et al,* 2000), with few reports of infection in fresh and processed pork (Siam *et al,* 1979) and about 13.3% *T. spiralis* infection among 1,025 rodents collected from and around abattoirs in Alexandria (Lotfy *et al,* 1999). Besides trichinosis was incriminated among seven zoonotic nematodes that affected human eyes (Alashry and Morsy, 2021).

The life cycle begins by the release of infective larvae in the stomach, which then invade the duodenum where they mature to female and male adult worms. Fertilized female laid newborn larvae and giving rise to the intestinal phase of infection. Thus, the larvae migrate mainly via blood stream to skeletal muscles to encyst and giving rise to muscular phase (Bruschi and Murrell, 2002).

In humans, infection could remain asymptomatic if it involves a low number of larvae, but in case of ingestion of few hundred larvae, gastrointestinal symptoms appear as soon as 2 days post-infection, followed by development of serious, rarely fatal, disease, but unlike in humans, in animals *T. spiralis* can reach a high worm burden without any clinical symptoms (Pozio and Zarlenga, 2013). So, larvae induces a complex immune response, which in human is better characterized by humoral rather than cellular responses, due to the importance of humoral response for diagnostic purposes (Pinelli *et al,* 2007).

T. spiralis is able to direct the immune system towards mixed T helper type 1 and T helper type 2 (Th1/Th2) immune responses with the initial predominance of Th1 response and the subsequent domination of Th2 type causing the gut pathology development (Ishikawa *et al,* 1998). It can also down-re-

gulates host immune response via the interfering with dendritic cell (DCs) maturation and signaling to increase the number of regulatory T-cells (Della *et al,* 2017).

In the intestinal phase of trichinosis, the drive of host immune response to a Th1 type depends on the type of signal from DCs, which represent a crucial link between innate and adaptive immunity. Worm antigens stimulate DCs maturation and histocompatibility complex class II expression on the cell surface, inducing the development of a Th1 immune response (Sher *et al*, 2003). Studies showed significantly up-regulation of Th1 cytokines such as IL-12, INF-γ, IL-1β & TNF-α during early stage of *T. spiralis* infection (Yu *et al*, 2013). INF-γ & IL-12 are of vital importance as they induce DCs maturation, enhances the development and differentiation of Th1 cells, up-regulate the expression of inducible nitric oxide synthase (iNOS) and increased the production of pro-inflammatory cytokines such as TNF-α, IL-1β & IL-6 play an important role in intestinal inflammatory response during trichinosis (Venturiello *et al*, 2007).

However, the exposure to *T. spiralis* antigens (TSL-1) increased IL-4 and IL-10 secretion from Th2 cells with a decrease in INF-γ production that shifting immune response to a strong Th2 type, which is protective and responsible for worm expulsion from the gut. The IL-10 is necessary for a successful intestinal immune response because the absence or decrease of cytokine causes a high susceptibility to the infection by *T. spiralis* and significant delay in the worm expulsion with subsequent increase in the parasite burden (Ilic *et al*, 2012).

No doubt, malnutrition is responsible for a great number of morbidity and mortality worldwide. In addition to disordered nutrient assimilation and inadequate food intake, malnutrition is also characterized by recurrent infections and chronic inflammation, implying an underlying immune deficiency. The complex of malnutrition and its overlapping comorbidities are poorly understood (Ahmed *et al*, 2014). Impairment of both innate and adaptive immune system has been consistently demonstrated in undernourished individuals (Claire *et al*. 2016).

Understanding host immune response during malnutrition is essential for novel therapeutic approaches and to support international goals to improve nutrition and wellbeing. Association between malnutrition and intestinal parasitic infection, particularly in individuals from developing countries was recognized for many decades. Accumulating evidences have shown that when both situations exist in the same person, there is a higher parasite burden as the result of an immune response defect (Ing *et al*, 2000).

In this context, since the discovery of glucocorticoids (GC) and their potent anti-inflammatory effects, they were widely used to control inflammatory and autoimmune diseases. But, their clinical efficacy is compromised by the metabolic effects of long term use as osteoporosis, dyslipidemia, hypertension, diabetes and immune system suppression (Barnes, 2011). GC exert immune-suppressants effect by regulating expression of genes encoding many pro-inflammatory cytokines (TNF-α, IL-1β, IL-12 & INF-γ) and inflammatory enzymes as inducible nitric oxide synthase, cyclooxygenase and inducible phospholipase (Agnes and Karen, 2011).

Glucocorticoids are indicated for treatment of intestinal inflammatory response in trichinosis (Gottstein *et al* 2009). However, its beneficial use is limited by the fact of treatment with GC suppresses the host immune system, favoring *T. spiralis* infection.

This study aimed to evaluate *T. spiralis* activity between malnutrition and well-nutrition experimentally infected Albino mice, to investigate the effect of GC long term use on intestinal immunity to infections.

Materials and Methods

Ethical considerations: The study was done at Theodor Bilharz Research Institute due to Guidelines with experimental animals.

Animals: Total of 28 clean laboratories bred Albino mice, 8-10 weeks old and weigh-

ed 25-30grams. They were divided into four groups: control non infected, infected well-nourished, infected mal-nourished and infected GC-treated mice. All mice received a control diet (20% casein) except mal-nourished ones that received a protein-deficient diet (6.5% casein). For immunosuppression, GC-treated mice were given dexamethazone intraperitoneally at a dose of 25ug/day for 3 weeks before infection. Immunosuppressed mice continued to receive dexamethasone throughout the experimental time (Rasmussen and Healey, 1992). All mice were housed in groups of two to three mice each in wire-floored cages in accordance with the institutional and national guidelines.

Trichinella spiralis were obtained from infected pork and maintained in the Parasitology Department at Theodore Bilharz Research Institute. *T. spiralis* larvae were harvested, and mice orally infected with 250larvae/mouse by pepsin-hydrochloric acid digestion method (Larsh and Kent, 1949).

Worm burden determination: Mice were sacrificed on 7^{th} (intestinal phase) and 21^{st} (muscular phase) day post infection to evaluate the efficacy of malnutrition and GC on the intestinal and muscular stages of trichinosis. For counting intestinal worm burden, the small intestine was dissected out, opened longitudinally, and then maintained (10cm long pieces) in a Petri-dish containing saline for 4 hours at 37^{o}. Then, intestines were discarded, and the worm was counted in the sediment by a stereomicroscopy. To count worms in muscle, diaphragm from each mouse was collected and digested in pepsin-Hcl at 37˚C overnight, parasite adults and larvae were harvested and counted under a microscope (Chu *et al*, 2016).

Cytokines assay: Blood samples from each mouse were collected via a small puncture of caudal vein by using a sterile needle. Sera were separated by centrifugation at 6000 rpm for 10 minutes. Serum concentrations of Th1 (IFN-y & IL-12), Th2 (Il-4&Il-10) and pro-inflammatory (TNF-α, IL-1β & IL-6) were determined by ELISA (Bio-source International, Camarillo, California, USA) as recommended by the manufacturers guides.

Statistical analysis: Data were collected, tabulated, and analyzed by using statistical package of social sciences program, version 18 (SPSS Inc. Chicago, Illinois, USA). Data were expressed as means and SD, analyzed using a two-tailed Student's t-test for unpaired means. Significant was considered if a P value less than 0.05.

Results

The results showed that at day 7p.i. (intestinal phase), adults counted from intestine of infected mal-nourished and infected GC-treated mice were significantly higher than that of the corresponding well-nourished ones. Infected mal-nourished and infected GC-treated mice didn't differ significantly in intestinal worm burden. Also, larvae from diaphragm of infected malnourished and infected GC-treated mice at day 21p.i. were significantly higher than that in infected well-nourished mice. As in adults, no significant differences were between mean number of encysted larvae in diaphragm of infected malnourished and infected GC-treated mice at day 21p.i.

Effect of *T. spiralis* infection on Th1 & Th2 immune responses in well-nourished mice showed mean levels of IFN-y & IL-12 increased in early infection at day 7p.i, but decreased later in muscular phase at day 21p.i. Th2 cytokines IL-4 & IL-10 was significantly lower than Th1 cytokines at day 7p.i, but, in day 21p.i. Th2 cytokines predominate were significantly higher than Th1 type. Levels of all cytokines were significantly higher than the corresponding non infected control. Effect of *T. spiralis* infection on Th1 & Th2 immune responses in malnourished mice showed that levels of INF-y & IL-12 were higher during intestinal phase than in muscular phase, and vice versa with Th2 cytokines. All cytokines concentrations in malnourished mice were significantly lower than in well-nourished mice.

Effect of GC long therapy on Th1 & Th2 immune responses against *T. spiralis* show-

ed that levels of all Th1 & Th2 cytokines were significantly lower in infected GC-treated mice than infected well-nourished mice at 7 & days 21p.i. But, cytokines concentrations were higher in sera from infected GC-treated mice than in infected malnourished mice without statistical significance. Pro-inflammatory mediators in normal mice showed low constitutive levels of TNF-α, IL-1β & IL-6 cytokines were in sera of normal uninfected mice, than in infected well-nourished mice. All sera from infected well-nourished mice were examined for TNF-α, IL-1β & IL-6 cytokines at day 7p.i. Levels of cytokines were significantly higher in infected well-nourished than in control mice.

Pro-inflammatory mediators in malnourished mice showed that sera mean levels were higher in infected malnourished than in control mice. Cytokines concentrations were significantly lower than in well-nourished mice. Pro-inflammatory mediators in infected GC-treated mice showed that mean levels of cytokines were significantly lower than in infected well-nourished animals at day 7p.i. Details were given in figures (1, 2, & 3).

Discussion

T. spiralis is a nematode that establishes a long-lasting infection in the skeletal muscles of its host and is transmitted by eating raw or undercooked meats contaminated with the parasite larvae (Gottstein *et al,* 2009). To establish the infection, the parasite has to modulate host's immune response to avoid their own destruction. The infection is characterized by the upregulation of a Th1 type of immune response at the beginning of the intestinal phase. A Th2 type of immune response, which is protective and responsible for the worm expulsion, becomes dominant at the time point when extensive larval dissemination takes place (Mosmann, 1991). There is accumulating evidence that malnutrition as well as GC treatment impair both innate and adaptive immunity with subsequent exacerbation of parasitic infections (Mohamed *et al,* 1983; Carman *et al,* 1992; Muñoz-Carrillo *et al,* 2017; Saracino *et al,* 2020; Vila *et al,* 2019; 2021). The present study revealed that counted adult worms from the intestine of infected mal-nourished mice were significantly higher than that obtained from the corresponding well-nourished ones at day 7p.i. In the same way, the mean number of isolated larvae from the diaphragm of the infected mal-nourished mice at day 21p.i. was significantly higher than that obtained from the infected well-nourished group. These results can be explained as the deficiency of the dietary protein can impairs mucosal immunity and impact negatively on Th2 immune responses that are required to control the helminthic infections (Koski and Scott, 2001). It was reported that IL-10 plays a significant role in the clearance of *T. spiralis* infection. The absence or decrease of that cytokine causes a high susceptibility to the infection and subsequently a significant increase in the parasite burden (Ilic *et al,* 2012). In cytokines assay, serum concentrations of Th1 (IFN-γ&IL12) and Th2 (IL-4&IL-10) cytokines were evaluated in infected well-nourished mice at particular days 7 & 21p.i, which corresponded the intestinal and muscular phases of infection respectively. The results indicated that Th1 response increased significantly at the beginning of infection, unlike Th2 cytokines that were higher at day 21p.i. The induction of Th2 immune response was crucial in worm expulsion and termination of infection (Ing *et al,* 2000; Vila *et al,* 2019; Saracino *et al,* 2020). In contrast to the observations with well-nourished mice, the production of Th1 & Th2 cytokines were severely impaired at all-time points of infection in infected mal-nourished mice. This may be explained by the fact that malnutrition retard the formation of vitally basic nucleic acids (DNA & RNA) and impair gene encoding for the host immune responses (Chu *et al,* 2016). This agreed with GC treated mice that showed Th1 &Th2 cytokines didn't increase significantly during infection, but increased in control and well-nourished mice. Pro-inflammatory mediators TNF-α, IL-1β

& IL-6 are produced from Th1 cells, CD4 + cells, macrophages, and dendritic cells that regulate cell activation and differentiation and homing of the immune cells to infection site to eradicate pathogens (Zhang and An, 2007). Elevated levels of pro-inflammatory cytokines were reported in intestinal *T. spiralis* phase (MuñozCarrillo *et al,* 2017). In the present study, effect of malnutrition and GC therapy on secretion of pro-inflammatory mediators during intestinal infection phase showed means levels of TNF-α, IL-1β & IL-6 significantly lower in infected malnourished & infected GC-treated mice as compared to infected well-nourished ones. Lacking these pro-inflammatory mediators negatively affect intestinal defense against *T. spiralis* infection.

Conclusion

Malnutrition and GC treated impair host immunity to *T. spiralis* infection. A healthy balanced diet is an important for maintaining good health and wellbeing. Glucocorticoids therapy must be done carefully to avoid their side effects and immunosuppression.

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Explanation of figures

Fig. 1a: Mean number of adults in intestine of mice groups, b: Mean number of *T. spiralis* larvae recovered from diaphragm of mice groups.
Fig. 2a: Serum concentrations of IFN-□, IL-12, IL-4 and IL-10 at different days of age, b: Cytokines profile in infected well-nourished mice, c: Cytokines profile in infected mal-nourished mice, d: Cytokines profile in infected GC-treated mice.
Fig. 3a: Infected well nourished, b: Infected mal-nourished mice, c: Infected GC-treated mice, d: Normal control mice