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Toxocara canis: Impact of preweaning nutritional deprivation on the pathogenesis of pneumonia in the mouse

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Abstract

The present study was conducted to better understand the immure response to *Toxocara canis* pneumonia in mice with preweaning nutritional deprivation. Breast-fed Swiss mice, undernourished due to large litter size (up to 15 pups) and paired controls with only 5–8 pups were used. At 21 days old, both groups were infected with *T. canis* larvae. Liver retinol, retinyl palmitate, and inflammatory infiltrate in lungs were compared in both groups. Significantly lower levels of retinol and retinyl palmitate in liver tissue confirmed the hypovitaminosis A ($P < 0.0001$ for both comparisons) in the nutritionally deprived animals. Histological analysis showed similar eosinophilic infiltration in both groups at day 3 but was significantly more severe in undernourished mice at day 20 post-infection ($P = 0.01$). The present findings indicate that preweaning undernourishment is associated with a more severe inflammation in response to *T. canis* pneumonia. It suggests that vitamin A deficiency that persists after nutritional rehabilitation, may contribute to the severity of *T. canis* infection. The authors suggest that nutritional status should be carefully investigated in patients with more severe clinical findings.

Keywords: Hypovitaminosis A; Undernourishment; Pneumonia; *Toxocara canis*

1. Introduction

Hypovitaminosis A, respiratory infection, and parasitosis are major causes of morbidity and mortality in developing countries, especially in children (Berman, 1991; Correa and Starke, 1998; Grupper, 1995; Shann, 1995). It is thought that the comorbidity of those conditions enhances the host vulnerability and contributes to treatment failure and higher mortality (Berman, 1991; Victoria et al., 1989).

Undernourishment may lead to a deficit of calories, protein or other nutrients such as vitamin A. This is well recognized to be associated with ocular, epidermal and respiratory dysfunction in early life (Kuming and Politzer, 1967; Zachman, 1995). It is also known that hypovitaminosis A impairs mucosal immune defenses (Wiedermann et al., 1993).

Some parasites that complete part of their life cycle in pulmonary tissue, induce inflammatory infiltration which may lead to respiratory complications. The nematodes *Strongyloides stercoralis*, *Necator americanus*, *Toxocara canis*, and *Ascaris lumbricoides* are the most frequently implicated (Allen and Davis, 1994; Amin and Wilnott, 1998; Gelpi and Mustafa, 1968).

The effects of early nutritional deprivation on anthropometric, metabolic, and immune parameters are known (Muralidhara and Shetty, 1986); however, its role in the presence of *T. canis* infection has not been studied previously.

To better understand the role of early undernourishment in the inflammatory response of *T. canis* pneumonia, the present study compared histopathological

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findings in lungs and tissue levels of vitamin A of preweaning undernourished and control mice during nutritional rehabilitation.

2. Materials and methods

2.1. Animals and treatment

Newborn Swiss mice from Central Vivarium of Ribeirão Preto Medical Faculty were used. In the large litter undernourished group, there were 4 litters of 15 siblings breast-fed by one mother each ($n=60$). In contrast, the control group had 4 litters of 5–8 siblings breast-fed by one mother each ($n=29$). This approach provided undernourished animals in the first group, as previously reported (Widdowson and McCance, 1960).

Body weight was measured at days 5, 10, 15, and 20 in both groups. All experimental procedures that used animals conformed The Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH 80-23) and local guidelines with regard to animal experimentation.

2.2. Toxocara canis eggs

Adult *T. canis* isolated from infected dogs were sexed by microscopy. Eggs were extracted from the uterus of females and were stored in 0.1% formol solution. The samples were filtered to remove nematode tissues, the eggs were re-suspended in 0.1% formol in Petri dishes, and examined daily. Eggs containing infective larvae were separated and prepared in doses of 10 eggs per microliter of 0.9% sodium chloride solution.

2.3. Toxocara canis infestation

Twenty-one-day-old Swiss mice in both groups received 200 eggs stage L2 by gavage. Five mice from each group were sacrificed on day 3, and 24 from control group and 36 from undernourished group were sacrificed on day 20. Lung and liver tissues were removed for further analysis.

2.4. Liver retinol and retinyl palmitate measurement

Liver tissues of mice euthanized after 20 days of infection were frozen at −20 °C and stored until the assay date. To confirm that undernourished mice were vitamin A deficient, liver samples were extracted using acetonitriledichloromethane-methanol (70:20:10, v/v/v). A column set with a guard-column packed with 10 μm Spheri-10 RP18 (3 cm × 0.46 cm) and a analytical stainless-steel column packed with Ultrasphere ODS (5 μm) (15 cm × 0.46 cm) (Beckman, San Ramon, CA) were used, hepatic retinol and retinyl palmitate were extracted and measured by HPLC (Kronton Instruments R, model T414) as previously described (Arnaud et al., 1991). Working standard solutions were processed in the same way. Percent recovery, as determined by adding known amounts of retinol to homogenates were 90–95%.

2.5. Lung histological analysis

The lung tissues obtained were fixed in 10% formalin, blocked in paraffin wax, and transferred to microscope slides for histological analysis after hematoxylin–eosin (HE) staining. Samples from undernourished and control groups were compared after day 3 and 20 of the *T. canis* infestation for severity and extension of inflammatory reaction in a blinded fashion by two observers using light microscopy, and graded as absent (0), low (1), moderate (2) or severe (3). Conflicting grades were re-evaluated until consensus was obtained. A weighted score considering equally severity and extension of the inflammation was applied for comparison.

2.6. Statistical analysis

Unpaired one-tail *t* Student's test was used to compare body weight, retinyl palmitate, and retinol levels. Fisher's exact test was used to compare the levels of lung inflammation between the groups.

3. Results

Undernourished mice presented with mean reduced growth of 31.25% compared to control group along of the first 20 days of life, as indicated by the body weight levels (Table 1).

Hepatic retinol and retinyl palmitate, representing the stores of body vitamin A were still significantly lower in the undernourished group at day 20 after *T. canis* infestation ($P<0.0001$ for retinol and $P<0.0001$ for retinyl palmitate) (Table 2).

Similar levels of moderate, predominantly eosinophilic, inflammatory infiltrate, was found in both groups of animals at the third day after *T. canis* infestation ($P>0.05$) (Figs. 1A and B, Table 3).

Table 1
Body weight (g) progression of undernourished and control mice (means ± SD) ($n=25$–47/group)

	Day 5	Day 10	Day 15	Day 20
Undernourished	$5.96 \pm 1.0^{*}$	$6.26 \pm 1.07^{\#}$	$6.69 \pm 0.88^{\#}$	$6.49 \pm 1.28^{\#}$
Control	6.76 ± 1.24	8.24 ± 1.24	9.38 ± 1.72	11.0 ± 1.98

Symbols represent significantly lower levels comparing undernourished and control groups, in the matched days, $^{*}P=0.0021$, $^{\#}P<0.0001$.