Downregulation of INF-Y and IL-10 in mice submitted to neonatal malnutrition and exposed to infection by S. mansoni

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Abstract
Newborn mice were breastfed by mothers fed with 17% casein diet (Nourished) or mothers fed with 8% casein diet (Malnourished). After weaning the animals received commercial normoproteic feed and were distributed thus: Nourished Infected, Nourished not Infected, Malnourished Infected and Malnourished not Infected. Infection was with 30 cercariae of S. mansoni. In the 14th week of infection, blood was collected for estimation of leukocytes and splenocytes culture performed to quantify IFN-y and IL-10. Maternal diet led to neonatal malnutrition which was accompanied throughout the experiment. Infection was associated to leukocytosis, neutrophilia and lymphocytosis, without difference between Nourished and Malnourished animals. Malnutrition leads to reduction of total leukocytes, neutrophils, monocytes, lymphocytes and basal levels of eosinophils. Eosinophilia was found in malnutrition associated with schistosomiasis. The malnutrition associated with schistosomiasis caused significant immunodeficiency in secretion of IFN-y and IL-10 when compared to eutrophic schistosomotic subgroup. Neonatal malnutrition associated with schistosomiasis alters the number of blood leukocytes and down-regulates IFN-y and IL-10 synthesis.

Introduction
It is well-known and common the association between schistosomiasis and malnutrition. However, studies associating schistosomiasis with malnutrition are sparse. The evolution of schistosomiasis and its clinical and laboratory manifestations are influenced by different factors, such as the immune status and nutritional condition. Malnutrition lead up to physiological complications, especially when it occurs during breastfeeding, since neonatal malnutrition has great impact on the immunological parameters that cause irreversible complications throughout life. In the chronic phase of schistosomiasis occurs a reduction of Th1 immune response, predominating Th2 immune response, that polarizes the synthesis of related cytokines, particularly IL-10, able to favorably modulate the inflammatory course. IL-10 negatively modulates the synthesis of Th1 cytokines, especially IFN-y, important for T cell proliferation and activation of macrophages. So, our study was to evaluate the levels of IL-10 and IFN-y in mice submitted to neonatal malnutrition and exposed to S. mansoni infection.

Material and Methods
Thirty-two females, Swiss webster mice, 24hrs after born were divided into two groups according to the maternal diet during breastfeeding. G1 - mothers fed with 17% casein diet (Nourished) and G2 - mothers fed with 8% casein diet (Malnourished). After weaning (22° days) the animals received Labina feed (Purina®) until the end of the experiments. Each group was divided into two subgroups according to exposure to Schistosoma mansoni: Nourished Infected (NI), Nourished not Infected (NNI), Malnourished Infected (MI) and Malnourished Not Infected (MNI). The infection was in 35th day of life with 30 cercariae per animal. Body weight was determined daily during breastfeeding and weekly after weaning. In the 14th week of infection the animals were anesthetized and blood collected by cardiac puncture and transferred into tubes with 3%-EDTA. The number of total leukocytes was obtained automatically and the number of neutrophils, lymphocytes, eosinophils and monocytes was estimated from blood smear. Subsequently, the animals were euthanized and spleen collected to perform splenocytes culture in RPMI 1640 supplemented. Suspensions of splenocytes (2.5x10^6 cells/0.5mL) were stimulated with Soluble Egg Antigen (SEA - 20μg/ml) and after incubation (72h, 37°C and 5% CO2) the supernatant was collected for dosing IL-10 and IFN-y cytokines. Cytokines were measured by enzyme-linked immunosorbent assay, ELISA Quanti-Kine®. All experimental procedure was approved by the Ethics Committee on Animal Use (CEUA)-UFPE (N° 23076.017352/2012-68). The statistical analysis was performed using the Student T test, Mann-Whitney test or Variance Analysis ANOVA.
Discussion and Conclusion
Malnutrition continues to be a health problem in Developing Countries. Parasitic infection like schistosomiasis has negative effects in nutritional status and nutritional deficiencies can lower the strength of the immune system. This is the first study to explore the association in an experimental model of neonatal malnutrition established through the breastfeeding by mothers with low protein diet. Previous studies showed that a low-protein diet during pregnancy and breastfeeding reduces offspring weight gain, causing weight loss from breastfeeding period to adulthood8. The weight gain seems to have not attenuated the reduction in body weight during schistosomiasis is competitive in regaining weight deficits obtained during the breastfeeding by mothers with low protein diet. In this study, malnourished mice infected with S. mansoni showed reduced synthesis of IFN-γ and IL-10 when compared to eutrophic schistosomotic subgroup. On the other hand, in a model of post-natal malnutrition, malnutrition leads to high levels of IL-10. Similar results were found in studies that only explore chronic schistosomiasis.11 In relation to levels of IFN-γ, Ishikawa et al (2009) observed reduction of IFN-γ in malnourished animals, but in the post-natal period. In a similar study design, Smith (2008) found no detectable levels of IFN-γ So, it is possible that our results, compared to IFN-γ and IL-10 are associated with neonatal undernutrition. Our study showed that neonatal chronic malnutrition associated with schistosomiasis alters the number of blood leukocytes and negatively regulates IFN-γ and IL-10 synthesis.

References