Schistosoma mansoni infection status and worm burden on cytokine expression

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ABSTRACT

It is recognized that gut inflammation and microbiome changes influence behavior and cognition through a "gut-brain axis". Billions of people have chronic gut inflammation due to helminths yet impacts of parasitism on the gut-brain axis remain largely unexplored. Schistosoma *mansoni* is a blood fluke that causes local inflammation in the large intestine and liver due to egg trapping. We hypothesize that this inflammation impacts microbiome composition, behavior, and cognition. Using a mouse model, we characterized systemic inflammation due to infection, analyzed microbiome changes and alterations in spatial learning and memory. Infected mice had significantly different inflammatory profiles than controls. Six of seven cytokines (IFN-γ, IL-1β, IL-4, IL-10, IL-17A, IL-23) showed significantly higher concentrations in infected mice. Considering worm burden, IL-10, IL-23, and IFN-γ concentrations had positive associations with worm burden (Wilcoxon rank sum test, P = 0.029, P < 0.001, and P = 0.003 respectively). IL-23 is strongly associated with severe immunopathology in schistosome infections, while evidence suggests that schistosomes can drive IL-10 production to suppress the immune response and immunopathology, perhaps suggesting a complex relationship between developing immunopathology and counter immune suppression. This study demonstrates that infected mice have distinct inflammatory profiles compared to control mice.

INTRODUCTION

Schistosomiasis is a neglected tropical disease that impacts people globally. It is surpassed only by malaria as the most harmful parasite (1). *Schistosoma mansoni* causes intestinal schistosomiasis. The worms reside in vessels of the large intestine. They release eggs which migrate through the intestinal wall and are then eliminated with feces. While infected, there are at least three phases: an acute Th1 response in weeks 3-5 followed by a Th2 response at weeks 5-6, and, if untreated, a Treg phase characterized by secretion of anti-inflammatory IL-10 and TGF-B (2). In the mouse model, schistosome infection modifies the composition of the gut microbiome to one associated with inflammation. This process causes disruption of the gut barrier and results in inflammation (2).

Study Objective:

Our central goal is to examine the links between inflammation from S. *mansoni* infection, gut microbiome changes during infection, and how this inflammation affects behavior and cognition. Our previous studies have shown that mice infected with schistosomes show altered behavior that indicate increased anxiety, increased compulsion, and delayed spatial learning and memory. Using a mouse model, our objective was to characterize systemic inflammation due to infection.

LIFE CYCLE and CLINICAL SYMPTOMS

Schistosoma mansoni transmission occurs via snails in infected water. Eggs are first shed by an infected human where they can then infect the snail. The infectious cercariae are released from snails and penetrate human skin, enter circulation and mature in the portal venous system. After mating, eggs are deposited in the mesenteric vessels. Eggs then cross the vasculature and subsequently the intestinal wall (3). Migrating eggs stimulate inflammatory granuloma formation and ultimately fibrosis. Egg penetration through the gut barrier leads to leakiness and the potential for modulation of intestinal or systemic immune system (2). Clinical symptoms of intestinal schistosomiasis involve abdominal pain, diarrhea and hematochezia, with serious cases involving hepatomegaly and ascites (1).

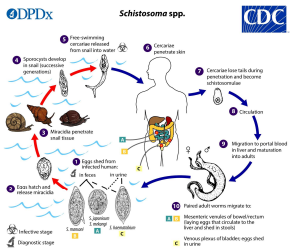

Figure 1: Life cycle of Schistosoma spp. Retrieved from: CDC (3)

STUDY DESIGN

A multiplex immunoassay was conducted to quantify seven serum cytokine measurements from 27 infected, 8 exposed but uninfected, and 12 control mice:

Th1: IFN-γ, IL-1β, TNF-α
Th2: IL-4 and IL-10
Th17: IL-17, and IL-23

Statistical analysis:

The statistical program R was used (4), including the package ggplot2 (5). Non-parametric Wilcoxon rank sum test and Kruskal-Wallis rank sum test were used to investigate effects of worm burden and of infection status, respectively, to compare their immune patterns.

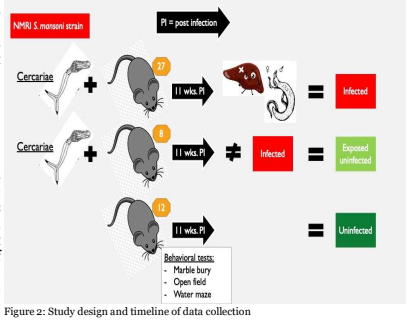

Figure 2: Study design and timeline of data collection

RESULTS

Infection status

Question: What is the effect of infection status on cytokine concentrations?

Hypothesis: We hypothesized that schistosome infection would increase inflammatory cytokines.

Conclusion: Six of seven cytokines (IFN-γ, IL-1β, IL-4, IL-10, IL-17A, IL-23) showed significantly higher concentrations in infected compared to uninfected mice (Kruskal-Wallis rank sum test, Fig. 3). No clear grouping pattern between Th 1, Th 2, and Th 17 was found.

Shainheit et al report that pathogenic Th17 response to schistosome egg antigen is dependent on IL-23 and IL-1β (5).

Could this ant-inflammatory cytokine be elevated in an attempt to dampen the damage that IFN-γ and IL-23 have?

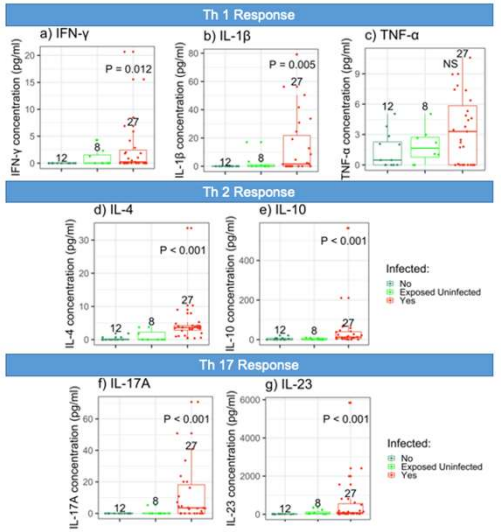

Figure 3: Summary of the uninfected (sea green), exposed uninfected (light green), and infected (red) mice for the systemic cytokine measures: a) IFN-γ, b) IL-1β, c) TNF-α, d) IL-4, e) IL-10, f) IL-17A, and g) IL-23. P-values represent significant values between infected and uninfected groups. Numbers represent sample sizes for each group.

Worm burden

Question: What is the effect of worm burden on cytokine concentrations?

Hypothesis: We hypothesized that increased worm burden would cause increase secretion of inflammatory cytokines.

Conclusion: IL-10, IL-23, and IFN-γ concentrations were the only cytokines that had a positive association with worm burden (Wilcoxon rank sum test, Fig. 4), which might suggest a complex relationship between developing immunopathology and counter immune suppression by these parasites.

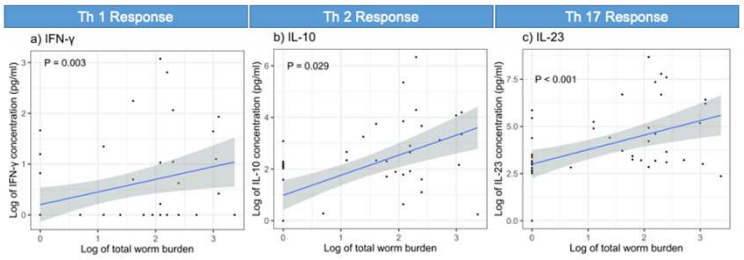

Figure 4: Summary of the worm burden for systemic cytokine measures: a) IFN-γ, b) IL-10, and c) IL-23. P-values represent significant association between worm burden and cytokine measure. Both axes are log transformed +1.

DISCUSSION

We found that schistosome infected mice had increased levels of most cytokines measured. Six of the seven cytokines (IFN-γ, IL-1β, IL-4, IL-17A, IL-23) showed significantly higher concentrations in infected mice when compared to control mice. Only TNF-α was not increased in infected mice. Three cytokines were correlated with worm burden (IFN-γ , IL-10, and IL-23). The immune response did not appear to be polarized to Th1, Th2, or Th17. This could be indicative of the mice entering the regulatory phase. It could also be that there is too much heterogeneity within the infection status groups that it is difficult to assign, although multivariate analyses suggests that this is not the case.

These data provide us with insight regarding the inflammation status of the infected mice; however, it is important to note that we only collected the cytokine concentrations at a single time point and analyzed this to investigate corresponding behavioral changes. It would be beneficial to analyze repeated measures of cytokines to strengthen our results. Lundy and Lukacs have done this to categorize infection stages, as seen in Figure 5 (6).

What remains unanswered is whether we are seeing systemic inflammation or tissue specific inflammation such as gut inflammation leading to these increases in cytokines. Schistosomiasis is a complex disease process that involves the host granulomatous immune response and the immunosuppressive capabilities of the parasite to prolonged its survival (7). This relationship needs to be investigated further.

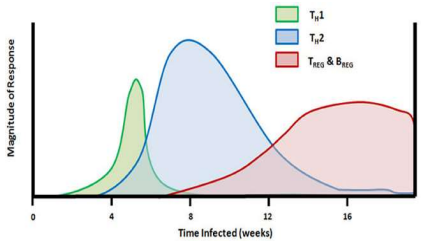

Figure 5: Timeline of stages of schistosome infection and predominating cell populations
Retrieved from: Lundy and Lukacs, Front. Immunol., 20 February 2013

CONCLUSION

Our future research will examine the links between inflammation, gut microbiome, behavior, and cognition. Furthermore, we will investigate how gut inflammation and microbiome changes affect brain derived neurotropic factor and inflammatory cytokines in brain regions to explore inflammatory effects secondary to S. *mansoni* on the gut-brain axis. The results will help us design experiments to help understand this system better and potentially translate this work to help understand the wide-ranging impact of S. *mansoni* on humans.

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