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PLASMA CYTOKINES DURING ACUTE HUMAN FASCIOLIASIS

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Los autores declaran no tener conflictos de interés.

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TABLA DE CONTENIDOS

RESUMEN

La fascioliasis es una trematodiasis de transmisión alimentaria con distribución mundial. Los niños menores de 15 años presentan la prevalencia más alta de infección. Hipotetizamos que la fascioliasis aguda está asociada a cambios más pronunciados en las citocinas en comparación con la enfermedad crónica o ausencia de infección por helmintos. Para probar esta hipótesis, se clasificó en 3 grupos a 33 niños que viven en zonas andinas de Perú: fascioliasis aguda, fascioliasis crónica, y sin infección por helminto. Se midió en plasma citocinas tipo Th1, Th2 y Th17 con matriz de microesferas citométrica. Niños con infección aguda presentaron niveles elevados de IL-5 e IL-17 comparado con controles ($p < 0.001$ y $p < 0.007$, respectivamente). El incremento de IL-5 plasmática en niños con infección aguda se asoció con la eosinofilia encontrada en ese grupo.

Palabras claves: Fasciola, IL-5, IL-17

ABSTRACT

Fascioliasis is a foodborne trematode endemic worldwide. Children under 15 years have the highest prevalence of infection. We hypothesized that acute fascioliasis would be associated with more pronounced cytokine changes than in chronic disease or no helminth infections. To test this hypothesis, 33 children who lived in the Peruvian highlands were classified into 3 groups: acute fascioliasis, chronic fascioliasis, and no helminth infection. Type Th1, Th2, and Th17 cytokines were measured in plasma by cytometric bead array. Children with acute infection had higher levels of IL-5 and IL-17 compared with controls ($p < 0.001$ and $p < 0.007$, respectively). The increased IL-5 plasma concentration in children with acute infection was associated with the eosinophilia found in that group.

Keywords: Fasciola, IL-5, IL-17

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IMMUNOLOGY AND HOST-PARASITE INTERACTIONS - SHORT COMMUNICATION

Plasma cytokines during acute human fascioliasis

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Abstract
Fascioliasis is a foodborne trematode endemic worldwide. Children under 15 years have the highest prevalence of infection. We hypothesized that acute fascioliasis would be associated with more pronounced cytokine changes than in chronic disease or no helminth infections. To test this hypothesis, 33 children who lived in the Peruvian highlands were classified into 3 groups: acute fascioliasis, chronic fascioliasis, and no helminth infection. Type Th1, Th2, and Th17 cytokines were measured in plasma by cytometric bead array. Children with acute infection had higher levels of IL-5 and IL-17 compared with controls ($p < 0.001$ and $p < 0.007$, respectively). The increased IL-5 plasma concentration in children with acute infection was associated with the eosinophilia found in that group.

Keywords *Fasciola* · IL-5 · IL-17

Introduction

Fascioliasis, caused by the foodborne trematodes *Fasciola hepatica* and *Fasciola gigantica*, is a zoonotic infection with a worldwide distribution. It is now regarded as an emerging disease in humans, since the number of reported cases is increasing (Robinson and Dalton 2009; Webb and Cabada 2018). The clinical presentation varies between acute fascioliasis and chronic fascioliasis. The acute presentation occurs during parasite migration through the liver and is characterized by right upper quadrant pain, fever, hepatomegaly, and marked eosinophilia, suggesting an active inflammatory response. The chronic presentation is associated with adult parasites in the biliary tree and often causes few symptoms. However, symptoms of biliary obstruction like colic with or without cholangitis, cholestasis, jaundice, or pancreatitis are characteristic of clinical cases of chronic *Fasciola* infection (Aksoy et al. 2005; Marcos Raymundo et al. 2002). However, most of those infected in endemic areas are subclinical and remain undiagnosed. In endemic countries such as Bolivia and Peru, children between 6 and 15 years are disproportionately affected by *Fasciola* (Lopez et al. 2012; Gandhi et al. 2019). Therefore, fascioliasis poses a threat for the development of children in endemic areas.

Few data are available regarding the immune responses to *Fasciola* infection. Most studies are from animal models. *Fasciola* parasites modulate the host immune system beginning early in the infection. Fascioliasis is associated with strong polarization to the Th2 and T-regulatory immune responses (Dowling et al. 2010; Graham-Brown et al. 2018). In contrast, *F. hepatica* excretory-secretory products suppress the Th17 and Th1 type immune responses to ensure parasite's survival (Dowling et al. 2010). The development of a strong Th1 response is associated with resistance to infection in animal models (Pleasance et al. 2011). By contrast, little is known about the human immune responses to *Fasciola*.

We hypothesize that acute fascioliasis is associated with pronounced cytokine activation. The purpose of this study is to characterize the cytokines profiles in asymptomatic

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children with evidence of acute *Fasciola* infection compared to children with chronic infection or no infection in a mesoendemic area of the highlands of Cusco in Southern Peru.

Materials and methods

Plasma samples were obtained from children that participated in a community-based study on the impact of *Fasciola* infection in the highlands of Cusco region in Peru (Cabada et al. 2018). The study enrolled apparently healthy children between 3 and 16 years old in rural communities and screened them with complete blood counts, Fas2 enzyme-linked immunosorbent assay (ELISA) for *Fasciola hepatica* antibodies (Bionoma SRL, Lima, Peru), and microscopy of three stool specimens for *Fasciola* and other helminth eggs. The parent study was approved by the Institutional Ethics Committee of Universidad Peruana Cayetano Heredia and the Institutional Review Board at University of Texas Medical Branch. Plasma from EDTA anticoagulated blood samples was stored at − 80 °C in a biorepository of de-identified specimens from children with parental consent for future use of biological samples. The repository is maintained at the University of Texas Medical Branch and Universidad Peruana Cayetano Heredia Collaborative Research Center in Cusco city, Peru.

Acute fascioliasis was defined as the presence of *Fasciola* antibodies by Fas2 ELISA associated with eosinophilia (> 600 cells/μL) with no evidence of *Fasciola* eggs in microscopy of three stool samples. Chronic fascioliasis was defined as the presence of > 100 *Fasciola* eggs/g of stool determined by the Kato-Katz test in the absence of other helminth eggs in three stool samples. Absence of helminth infection was defined as negative microscopy of three stool samples with negative Fas2 ELISA and no peripheral blood eosinophilia. Acute fascioliasis samples were matched by children's age (± 2 years), sex, and, when possible, by community of residence with samples from children in the other two groups.

IL-2, IL-4, IL-6, IL-10, TNF-α, INF-γ, and IL-17 protein levels in plasma samples were measured by cytometric bead array (BD Biosciences, San Jose, CA) in the Immunology Laboratory of the Alexander von Humboldt Tropical Medicine Institute, Lima, Peru. In addition, IL-5 plasma concentrations were measured by ELISA (BD Biosciences, San Diego, CA) following the manufacturer recommendations.

For statistical analysis, we performed non-parametric assays. IL-2, IL-4, IL-6, IL-10, TNF-α, INF-γ, IL-17, and IL-5 concentrations were compared between the two groups using the Mann–Whitney test. In both assays, a value of $p < 0.05$ was considered significant.

Results and discussion

Eleven plasma samples from subjects with acute fascioliasis were retrieved from the biobank for testing. The diagnosis was based on detection of specific antibody to *F. hepatica*, using an assay with good specificity. A high eosinophil count was also part of the case definition. The latter can be caused by a number of invasive helminths. However, the current studies were conducted in an area at high elevation with temperatures less conducive to organisms that must survive in soil. Thus, these areas have a low prevalence of soil-transmitted nematodes, less than that of fascioliasis. In addition, the children in that region are treated twice yearly with albendazole. Thus, the positive predictive value of the *Fasciola* FAS-2 ELISA in this region is quite high. Stool samples from three of these subjects had other helminth eggs. One had *Trichuris trichiura* and hookworm, one had both *Ascaris lumbricoides* and *Hymenolepis nana*, and one had *Hymenolepis nana*. The cytokine levels in those subjects were not different than others with just acute fascioliasis. Eleven samples from matched subjects with chronic fascioliasis and eleven samples from matched subjects with no gastrointestinal helminth infections were also selected from the biorepository. Samples from two subjects (one with acute and one with chronic infection) were insufficient to perform the IL-5 ELISA test.

The median age and eosinophil count of subjects whose samples were selected for testing are shown in Table 1. There were no significant differences in the levels of IL-2, IL-4, IL-6, IL-10, TNF-α, and INF-γ between the groups (Mann–Whitney test, $p > 0.05$). There were significant differences in IL-5 and IL-17 levels between groups (IL-5 $p = 0.0007$ and IL-17 $p = 0.0066$, Mann–Whitney test).

Table 1 Median age, eosinophil count, IL-5, and IL-17 levels among children in each group

	Age in years median (IQR*)	Eosinophils (cells/μL) median (IQR)	IL-5 (pg/mL) median (IQR)	IL-17 (pg/mL) median (IQR)
Acute fascioliasis	10.5 (7.3–11.5)	1,450 (925–2,400)	1.67 (0.53–3.66)	2 (0–5)
Chronic fascioliasis	10.2 (8.2–11.3)	200 (200–275)	0.0042 (0.00–0.095)	0
No helminths	10.7 (6.7–11.7)	100 (100–200)	0.019 (0.00–0.913)	0

*IQR interquartile range

We compared the plasma cytokine levels between children with acute and chronic *Fasciola* infections using a cytometric bead array and ELISA. Only IL-5 and IL-17 were found at higher plasma concentrations in samples from subjects with acute fascioliasis compared with controls. Interestingly, the levels of IL-5 and IL-17 were actually lower in those with chronic fascioliasis than in the controls, but this was not statistically significant. This may have been due to immunomodulation, which is characteristic of chronic helminth infections.

The higher levels of IL-5 detected in samples from children with acute infection compared to those with chronic infection are not surprising. Eosinophilia was part of the case definition and IL-5 has a critical role in generating an eosinophilic response (Klion and Nutman 2004; Yasuda and Kuroda 2019; Shin et al. 2009). In the cases of acute fascioliasis, this likely represents the acute cytokine response during the migration of the juvenile *Fasciola* through the liver that is characteristic of the acute fascioliasis presentation. Eosinophils are elevated in many helminth infections, especially in those with tissue-migratory stages (Yasuda and Kuroda 2019; Shin et al. 2009). Eosinophils have shown to have a key role in parasite killing activity in vitro (Klion and Nutman 2004). We previously demonstrated that eosinophils in acute fascioliasis demonstrate a proteomic profile suggestive of activation (Straub et al. 2011).

The higher levels of IL-17 detected in samples from children with acute infection compared to those with chronic infection are a novel observation. IL-17 has been associated with eosinophilia in childhood asthma (Mansour et al. 2017; Wakashin et al. 2009). This effect was thought to be mediated via GMSCF, which prolongs eosinophil survival. Thus, IL-17 may contribute to the eosinophil response in acute fascioliasis. However, literature reports that *F. hepatica* infection seems to suppress the Th17 immune response, both in acute and chronic stages, and there is a strong correlation between Th1/Th17 immune responses and the development of severe inflammation-mediated pathology in infected mice (Dowling et al. 2010). Further studies are required to confirm the role of IL-17 in acute infection in children living in endemic areas of fascioliasis.

In conclusion, IL-5 plasma concentration was higher in children with acute infection compared to controls. The role of IL-17 elevation in children with acute *Fasciola* infection is unclear and further studies are needed to elucidate if it has a protective or immunomodulatory role.

Author contribution MM and MMC conceived this study. The samples were collected and banked from a prior study initiated by MMC and ACW. Cytokine measurements were performed by CAS in the laboratory of MM. All authors were involved in data analysis, drafting, and revising the manuscript.

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Data availability Data are available upon request.

Declarations

Ethics approval and consent to participate The parent study was approved by the Institutional Ethics Committee of Universidad Peruana Cayetano Heredia and the Institutional Review Board at University of Texas Medical Branch, including collection and de-identified banking of specimens from children with written parental consent for future use of biological samples.

Conflict of interest The authors declare no competing interests.

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