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TITLE

CHANGES IN THE DISTRIBUTION PROFILE OF INTESTINAL BACTERIA IN SCHISTOSOMOTIC C57BL/6 MICE FED A HIGH-FAT DIET: AN ANALYSIS BY FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

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ABSTRACT

There is increasing evidence that the concomitance between schistosomiasis mansoni and obesity aggravates liver, splenic and intestinal tissue by modifying the architecture and physiology of these organs. Studies have supported the role of the mammalian gut microbiota in hepato-intestinal schistosomiasis and open the way to understanding the complex relationships between helminths, gut microbiota, host immunity and the pathophysiology of infection in the acute and chronic phases. Therefore, using a model of diet-induced obesity (DIO), we sought to understand this association. C57BL/6 male mice fed either a high-fat diet (60% fat) or a standard diet (10% fat) for 13 weeks was infected with 100 *Schistosoma mansoni* cercariae (BH strain). Mice were allocated into four groups: USC (uninfected fed standard diet), UHFC (uninfected fed high-fat diet), ISC (infected fed standard diet) and IHFC (infected fed high-fat diet). The development of obesity was assessed by blood lipid profile, including TC (total cholesterol), TG (triglyceride levels), LDL-C (low-density lipoprotein cholesterol), HDL-C (high-density lipoprotein cholesterol) and VLDL-C (very low-density lipoprotein cholesterol) and glucose concentrations, OGTT (oral glucose tolerance test), body mass and adiposity index. Mice were euthanized by CO₂ exposure 9 weeks after infection. Histological sections of the jejunum were stained with hematoxylin and eosin (H&E). For fluorescence in situ hybridization (FISH), sections of the mice's jejunum were collected and fixed in 10% buffered formalin for 48 hours. The material was incubated in 10% and 30% sucrose at 4°C for 24 hours for inclusion in OCT (Optimum Cutting Temperature) gels and subjected to rapid freezing in liquid nitrogen. FISH was performed on each slide using the conditions and buffers using 30% formamide in the hybridization buffer and probes for eubacteria (Thermo Fisher Scientific). IHFC showed a reduction in lipid profile, an increase in HDL-c, and better oral glucose tolerance, as well as a reduction in body mass. The results obtained from fluorescence in situ hybridization suggest the occurrence of bacterial translocation in the infected groups, especially in the group subjected to the high-fat diet. FISH revealed a higher concentration of bacteria around the *S. mansoni* egg and in the apex of the mucosal layer in ISC mice, while in the IHFC group we found a distribution of bacteria in the mucosal layers, especially in the crypts of Lieberkühn, the submucosa and the muscle, suggesting a possible translocation of bacteria in IHFC mice. This process is known to occur due to increased permeability of the intestinal mucosa, stimulated by the diet, increased bacterial growth, a weakened immune system and lesions caused by the *S. mansoni* egg during its passage into the intestinal lumen. Taken together, our data suggest that concomitant obesity and acute schistosomiasis have a significant impact on the architecture of the jejunum in C57BL/6 mice.

KEYWORDS

Schistosoma mansoni; Obesity; Gut Microbiota; Mice

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