Short Report: Human Schistosomiasis Is Associated with Endotoxemia and Toll-Like Receptor 2- and 4-Bearing B Cells

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Abstract. Schistosomiasis is caused by parasitic trematodes. Individuals can accumulate hundreds of intravascular worms, which secrete a myriad of antigenic molecules into the bloodstream. Some of these molecules suppress immunity to microbial Toll-like receptor (TLR) ligands, such as lipopolysaccharides, which may increase host susceptibility to coinfected pathogens. We show that schistosomiasis is associated with extremely high levels of endotoxemia as well as high mobility group 1, an endogenous inflammatory TLR ligand, in the absence of other coinfected pathogens. Circulating B cells express surface TLR2 and TLR4, reflecting systemic exposure to microbial ligands. Bacterial translocation may occur with schistosomal egg movement from the vascular to the gut and other routes, such as the skin during infection. Our report suggests that immunosuppressive schistosome antigens may have evolved to curb inflammatory responses to the high antigenic burden of translocated bacteria products and endogenous TLR ligands that arise during parasite exposure and inflammation.

Human schistosomiasis is a chronic inflammatory disease caused by intravascular parasitic trematodes. By day 2 post-infection in animal models, worms have entered the vasculature, where they have been shown to remain for up to 10 years in the human host secreting a myriad of molecules into the bloodstream, which modify multiple host cellular pathways. Individuals hyperexposed to parasitic schistosomes can accumulate hundreds of worms in their blood and thus, carry a significant antigenic burden. In particular, schistosomes produce several Toll-like receptor (TLR) ligands, including those for TLR2, TLR4, and TLR3. Evidence suggests that many schistosome-derived molecules, particularly TLR4 ligands and several glycoproteins, are immunosuppressive and down-regulate Th1 responses while actively promoting Th2-like immunity. For example, Schistosoma mansoni soluble egg antigens (SEAs) inhibit the activation of murine dendritic cells in response to lipopolysaccharide (LPS), CpG, and poly-I:C. Sm16 secreted by S. mansoni during skin penetration exerts potent inhibitory effects on the cytokine response to LPS and the exogenous TLR3 ligand, poly(I:C). SEA also suppresses LPS and poly-I:C-mediated maturation and production of interleukin (IL)-12, IL-6, and tumor necrosis factor (TNF)-α by human dendritic cells. These observations support the hypothesis that schistosomiasis increases susceptibility to coinfected pathogens. However, immunosuppression by schistosome antigens may also be clinically relevant in the context of blood-borne non-pathogenic bacteria. Chronic inflammatory diseases, including inflammatory and primarily metabolic or infectious diseases, are associated with increased translocation of bacteria into the bloodstream, which can lead to unremitting stimulation of the immune system. Chronic exposure to systemic bacteria can result from mucosal ulceration of the gastrointestinal tract in inflammatory bowel disease (IBD) or altered intestinal immune responsiveness, such as in progressive human immunodeficiency virus (HIV) disease. A disturbed microcirculation and edematous gut wall in heart failure also allows bacteria to enter the bloodstream. Any of these mechanisms could occur in schistosomiasis because of altered circulation from eggs lodging in the vasculature, disrupted gut epithelium and endothelium from egg translocation, or inherently suppressed immunity from schistosomiasis itself or coinfections, such as HIV. In addition, individuals at risk, and particularly hyperexposed populations, may also be exposed to bacterial or other microbial antigens within infested water or their skin microflora, both of which might be transported in with infecting cercaria. Microbial TLR ligands also have the propensity to release a host of endogenous TLR stimulatory ligands. These conditions set the host up for the accumulation of a myriad of systemic schistosome, microbial, and host-derived antigens.

We previously showed that chronic disease is associated with activated TLR2- and TLR4-expressing B cells in the periphery. These activated B cells can secrete significant amounts of cytokines and chemokines while in circulation. However, systemic host- and microbial-derived TLR ligands may modify B-cell responses in a pro- or anti-inflammatory manner. This has been shown primarily by the differential responses of human B cells to TLR4 ligands. Interestingly, in patients with chronic disease, persistent endotoxemia does not necessarily associate with systemic inflammation and poor health. This may be related to the type of LPS in the bloodstream. The number of acyl chains on lipid A, the inflammatory mojety of LPS, can dictate whether LPS is agonistic or antagonistic. For example, lipid A from Escherichia coli is hyperacylated with six acyl chains that mediate inflammatory pathways through TLR4. In contrast, lipid A from various species of commensal bacteria may be hypoacylated and act in an anti-inflammatory manner. In fact, in Crohn’s disease (CD), systemic LPS may be immunosuppressive for B cells through a reduced systemic LPS lipid A acylation burden.

We sought to determine if B cells were modified in human schistosomiasis and the potential effect of this disease on measurable TLR ligands in the bloodstream. We assessed the systemic TLR ligand burden and TLR expression by circulating
B cells in a cohort of multiply treated occupationally exposed car washers and fishermen in Western Kenya. This study was approved by the Scientific Steering Committee of the Kenya Medical Research Institute, the National Ethics Review Board of Kenya, and Boston University Institutional Review Board and was performed in western Kenya along the shores of Lake Victoria as previously described. Study participants (N = 47 total) were aged 21–63 years (mean age ± standard deviation = 35.6 ± 12.5 years) and considered hyperexposed to *S. mansoni* because they stand in the water for several hours a day either washing cars or fishing. Eggs per gram of feces (EPG) ranged from 0 to 2,880 (mean EPG ± standard deviation = 308 ± 664 EPG). Uninfected Kenyan subjects were recruited from the Kenya Medical Research Institute (N = 5). Uninfected/unexposed subjects (N = 50) were recruited from Boston, MA, as previously described. On informed consent, all blood samples were drawn into endotoxin-free, non-reactive heparin-containing tubes (BD Vacutainer with sodium heparin #366480; Becton Dickson, San Jose, CA) (Figure 1A). Plasma was stored at −80°C until use. Whole blood was evaluated by flow cytometry for expression of TLR4 and TLR2 on circulating CD19 + B cells. Briefly, 100 μL of blood were incubated with fluorescently labeled antibodies (anti-CD19) purchased from BD Pharmingen (San Jose, CA) and eBioscience (San Diego, CA; anti-TLR2 and anti-TLR4). Red blood cells were lysed with FACS Lysis Buffer (BD Pharmingen). Assessment of surface expression on B cells was performed with gates generated with anti-CD19 plus the appropriate isotype controls for each sample. Endotoxin was measured in the plasma with limulus amebocyte lysate (LAL) assay (Lonza, Basel, Switzerland) as previously described for other chronic inflammatory diseases. Plasma high-mobility group box 1 (HMGB1) levels were measured by enzyme-linked immunosorbent assay (ELISA; Shino-Test Corporation, Kanagawa, Japan). Stool samples were examined for *S. mansoni* eggs and other helminth ova by the modified Kato–Katz method (Vestergaard Frandsen; two slides each from three stool specimens obtained over several days). Subjects positive for *S. mansoni* were treated with 40 mg/kg praziquantel, and those positive for other helminth ova were treated with 400 mg of albendazole as previously described. Although some study participants were schistosome egg negative at the time of blood draw (n = 5), they were actively exposed to infective cercaria in Lake Victoria. Sample sizes of readouts varied because of incomplete availability of certain subject data.

We found that hyperexposure to infectious schistosomes was associated with a high level of systemic endotoxin (Figure 1A). Significantly, levels of endotoxin were 10 times higher than the levels reported in lethal septic shock. This suggests that schistosome antigens can suppress the host response to the large dose of systemic LPS. Alternatively, the lipid A acylation burden may be reduced in schistosomiasis with an increased prevalence of hypoacylated LPS. Schistosome egg translocation from the mesenteric blood vessels to the gut lumen plays a role.

![Figure 1](https://example.com/image1.png)

**Figure 1.** Schistosomiasis is associated with a high TLR ligand burden. (A) Plasma endotoxin levels are increased in occupationally hyperexposed individuals (N = 36) compared with uninfected/unexposed North Americans (N = 15; P < 0.0001) and uninfected Kenyans (N = 5). Blood collection tubes rinsed with phosphate-buffered saline (PBS) did not induce a false-positive reaction on the limulus amebocyte lysate (LAL) assay (N = 3). Polymyxin B-treated SEAs do not react with the LAL assay (N = 3; separate measures). (B) Levels of plasma endotoxin were not associated with fecal egg positivity in hyperexposed subjects: egg negative (N = 5); egg positive (N = 13); P = 0.805. Data are expressed as box plots, with the horizontal lines representing the 25th, 50th, and 75th percentiles. (C) Plasma high-mobility group box 1 (HMGB1) levels are elevated in hyperexposed subjects (N = 18) compared with uninfected/unexposed subjects (N = 8; P = 0.0006). (D) Plasma HMGB1 levels were not associated with fecal egg positivity in hyperexposed subjects: egg negative (N = 5); egg positive (N = 13); P = 0.621. Data are expressed as box plots, with the horizontal lines representing the 25th, 50th, and 75th percentiles. (E) There is no correlation between plasma endotoxin and HMGB1 levels in hyperexposed subjects (N = 18; r = −0.22; P = 0.365). Mann–Whitney U Test was used for differences in means and linear regression for correlation.
in systemic bacterial translocation in mice.\textsuperscript{26} We obtained EPG data on 18 of the study participants but found no difference in endotoxin levels between egg-positive and egg-negative subjects hyperexposed to schistosomes (Figure 1B). It is also possible that the LAL assay may detect blood-borne schistosome antigens. However, polymyxin B-treated egg antigens (gift from Edward J. Pearce, Trudeau Institute, Saranac Lake, NY) did not react with the assay (Figure 1A). Thus, a significant amount of endotoxin may enter the blood through other routes, such as the skin during cercarial penetration, and the anatomical site of bacterial origin might play a role in the inflammatory potential of LPS.\textsuperscript{17}

LPS and other inflammatory mediators have been shown to induce the release of HMGB1.\textsuperscript{18} HMGB1 is an alarmin that mediates effects through its receptor for advanced glycation end product (RAGE) and has been implicated in the pathogenesis of septic shock.\textsuperscript{27,28} HMGB1 can also contribute to inflammation through TLR2, TLR4, and TLR9.\textsuperscript{29,30} Plasma HMGB1 levels were also quite high in the exposed cohort compared with unexposed/uninfected and were three times the level in IBD.\textsuperscript{25} Because SEAs can dampen responses of cells to LPS,\textsuperscript{31} we predicted that HMGB1 levels would be lower in egg-positive subjects. However, there was no difference between egg-positive and egg-negative study subjects, although fecal counts do not take into account liver egg or infecting cercariae burdens. The lack of a positive correlation between LPS and HMGB1 (Figure 1E) suggests that LPS in schistosomiasis does not promote the release of HMGB1 from cells; however, the data are cross-sectional. This may be suggestive of the LPS having characteristics of antagonistic lipid A or additional evidence that schistosomiasis has an immunomodulatory effect to the relatively large TLR ligand burden in the blood.

Because B cells can recirculate, TLR expression on peripheral blood B cells can reflect the inflammatory state of a patient as receptor levels may correlate directly with disease activity.\textsuperscript{32} Furthermore, schistosomes reside in the bloodstream and thus, can affect circulating cellular activation levels. We found that B cells expressed high levels of both TLR4 (Figure 2A and B) and TLR2 (Figure 2C). Thus, B-cell phenotypes are altered and resemble those from non-infectious chronic inflammatory diseases with high systemic levels of TLR ligands.\textsuperscript{19,22} However, in contrast to TLR2, B-cell TLR4 is not necessarily inflammatory and can function to suppress cytokine production through different TLR4 ligands than to which myeloid cells respond.\textsuperscript{22} Furthermore, this effect is disease-specific as B cells from patients with chronic inflammatory disease may respond to different TLR4 ligands.

We show that schistosomiasis is associated with a high antigenic TLR ligand burden. The lack of relationship between LPS and HMGB1 suggests that, at least at the level of endotoxin and probably at the level of schistosome antigens, the TLR4 ligand burden in schistosomiasis has a null or antiinflammatory effect. Antiinflammatory LPS may come from specific species of bacteria or from host modification of lipid A, such as from neutrophil enzymes.\textsuperscript{32} Another intriguing possibility is that schistosomes themselves modify acyl chains of LPS, because they have been shown to alter acyl chains on phosphatidyl serine, to produce yet another array of TLR ligands.\textsuperscript{33} In contrast, HMGB1 can stimulate B cells to secrete IL-8 through TLR2 in CD and is likely inflammatory.\textsuperscript{22} Additional work needs to be done to determine its receptors and cellular distribution in schistosomiasis.

\textbf{In conclusion, our report suggests that schistosomes may have evolved mechanisms to control host inflammation to other microbial antigens during infection. Understanding the full spectrum of the host immune response in schistosomiasis is critical to improving the health of people at risk for this disease. Our results may also open the door for the discovery of effective treatments for diseases that are associated with inflammatory endotoxin, such as septic shock or type 2 diabetes. In fact, cystine proteases secreted from \textit{S. mansoni} and another trematode parasite, \textit{Fasciola hepatica}, protect mice from LPS-mediated septic shock by suppressing the release of nitric oxide, IL-6, TNF-\textgreek{c}, and IL-12 from macrophages, showing the array of effects that schistosome antigens have on host immunity.}\textsuperscript{14}

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