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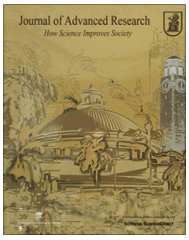

ORIGINAL ARTICLE

Upregulation of Toll-like receptor 2 and nuclear factor-kappa B expression in experimental colonic schistosomiasis

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ABSTRACT

Role of different mediators was described in the development of the granulomatous response and fibrosis observed in intestinal schistosomiasis. However, both Toll-like receptor 2 (TLR2) and nuclear factor kappa B (NF-κB) have not yet been investigated in intestinal schistosomiasis. This study aimed to characterize the role of TLR2 and NF-κB in the pathogenesis of intestinal schistosomiasis. Experimental animals were divided into two groups; group I: non-infected control group and group II: mice infected subcutaneously with *S. mansoni* cercariae. Colon samples were taken from infected mice, every two weeks, starting from the 6th week postinfection (PI) till 18th week PI. Samples were subjected to histopathological and immunohistochemical studies. Colon of *S. mansoni* infected mice showed histopathological changes in the form of mucosal degeneration, transmural mononuclear cellular infiltration and granulomas formation. Immunostained sections revealed significant increase in TLR2 and NF-κB positive cells in all layers of the colon, cells of the granuloma and those of the lymphoid follicles 10 weeks PI. All these changes decreased gradually starting from 12 weeks PI onward to be localized focally at 18 weeks PI. In conclusion, recruitment and activation of inflammatory cells to the colonic mucosa in intestinal schistosomiasis are multifactorial events involving TLR2 that can trigger the NF-κB pathways. Hence, down-regulation of both TLR2 and NF-κB could be exploited in the treatment of colonic schistosomiasis.

Introduction

Intestinal schistosomiasis is the most common manifestation of infection with *Schistosoma mansoni* in endemic areas and if not diagnosed and treated early, it might lead to complications such as hepatosplenic schistosomiasis, which have high morbidity and mortality, and chronic intestinal schistosomiasis which may be presented by severe rectal bleeding or intussusceptions, pericolic or mesenteric granuloma, and intestinal obstruction [1].

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This disease is caused mainly by the host's immune response to schistosome eggs. The granulomas formed around the eggs aim to sequester or neutralize pathogenic egg antigens and also lead to fibrogenesis in host tissues [2]. Chronic morbidity in schistosomiasis develops when schistosome eggs lodge in the gut, liver and other organs causing extensive tissue damage. Immune responses to schistosome antigens manifest a striking shift from a moderate Th1 to a Th2-dominated response with the onset of egg laying around 5–6 weeks which is responsible for fibrosis and much of the pathology [3,4].

Different mediators have been described to play a critical role in the development of the granulomatous response and the resulting fibrosis observed in schistosomiasis, e.g. IFN-γ, IL-10 [5] and TNF-α [6]. However, the role of TLR2 and nuclear factor (NF)-κB has not yet been investigated in intestinal schistosomiasis.

NF-κB is a transcription factor that regulates some processes such as inflammation, apoptosis, stress response, wound healing and angiogenesis [7]. NF-κB is markedly activated in the inflamed gut, especially in macrophages and epithelial cells. Sustained activation of NF-κB is detected in the intestinal lamina propria to the point that the degree of NF-κB activation correlates with the severity of intestinal inflammation [8].

Toll-like receptors (TLRs) belong to a family of receptors that can recognize all classes of pathogens, including parasitic invaders. TLRs are thought to play an important role in the rapid activation of innate immune responses in coordination with the adaptive immune response to eliminate pathogens [9]. Toll-like receptors are predominantly expressed on immune related cells such as monocytes, macrophages, neutrophils, dendritic cells, lymphocytes, and NK cells [10]. Moreover, it has been shown that TLRs are also expressed on other non-immune cells, especially in the epithelium, including epithelial cells of the gastrointestinal and respiratory tracts [11,12]. The link between the activation of TLR2 and intestinal disease has been reported, both in the colon and in the ileum [13].

Whether TLR2 and NF-κB are involved in the pathogenesis of intestinal schistosomiasis is still to be elucidated. Their role to induce cellular activation and the mechanisms by which they can affect the pathogenesis of intestinal schistosomiasis needs to be studied.

Material and methods

Parasite

Laboratory bred *Biomphalaria alexandrina* snails were purchased from the Schistosome Biological Supply Program, Theodore Bilharz Research Institute (Giza, Egypt). According to Lewis et al. [14], the snails were placed in beakers containing dechlorinated water (1 ml/snail) and exposed to direct light at 28 °C for at least 4 h. *S. mansoni* cercariae shed from the snails were used to infect the experimental animals of the study. The cercarial suspension was adjusted to contain 50–60 cercariae/0.1 ml dechlorinated water.

Animals and experimental design

A total of 115 laboratory bred male Swiss albino mice, 6–8 weeks old, weighing 20–25 g were purchased from Theodore Bilharz Research Institute (Giza, Egypt). *The experiment was conducted in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978)*. Mice were housed in appropriate cages and allowed ad libitum for a commercial rodent chow and tap water. They were divided into two groups; group I: 10 non-infected mice (control group) sacrificed once 6 weeks from the start of the experiment, group II: mice infected by subcutaneous injection of 0.1 ml cercarial suspension as described by Peter and Warren [15] then 15 mice were sacrificed every two weeks postinfection (PI) starting from 6 week PI till 18 week PI. From each mouse, colon was removed and preserved for histopathological and immunohistochemical studies.

Histopathological studies

The distal 3 cm of the colon was cut and washed with saline and fixed in 4% formol saline. The specimen was dehydrated in ascending grades of ethanol and cleared in xylene then embedded into paraffin. Serial sections from colonic tissues of 5 μm thickness were obtained and stained with hematoxylin and eosin (H&E). Four colonic sections were examined for each mouse [16]. Histological score in these sections was determined according to Dieleman et al. [17] with modifications. The following items were assessed: the degree (0–3) and extent (0–3) of inflammation, crypt damage (0–4) and the area involved (0–4) (Table 1). The score of each parameter was multiplied by four and the sum of these multiples was the final score. For the estimation of the number and size of granuloma, morphometric analysis was performed using Leica microscope with built-in camera (Leica Image System Ltd, Cambridge, UK) in Histology Department, Faculty of Medicine, Tanta University to assess the mean number and size of granuloma in five randomly selected fields at 400× magnification [18].

Immunohistochemical staining

Briefly, paraffin-embedded sections were deparaffinized, rehydrated, and microwave heated for 15 min in 0.01 mol/L citric buffer (pH 6.0) for antigen retrieval. Then, 3% hydrogen peroxide was applied to block endogenous peroxidase activity. After 30 min of blocking with normal serum (Invitrogen, Carlsbad, CA), the primary antibodies were added and incubated overnight at 4 °C. They were in the form of TLR2 (a mouse monoclonal antibody; Dako) and the primary rabbit anti-phospho-NF-κB p65 ser276 antibody (NF kappa B p65) (henceforth pp65, Cell Signaling, Danvers, MA). Slides were washed thrice with phosphate buffer solution (PBS), each for 5 min. The biotinylated secondary antibody and the streptavidin–biotin complex were applied, each for 60 min incubation at room temperature. After rinsing with PBS, the slides were immersed for 10 min in 3,39-diaminobenzidine (Sigma, St. Louis, MO) solution (0.4 mg/mL with 0.003% hydrogen peroxide), monitored under the microscope and the reaction was terminated by adding distilled water. Slides were then counterstained with hematoxylin, dehydrated, and coverslipped. Immunoreactivity of NF-κB appeared as brown cytoplasmic and nuclear staining of varying degrees of intensity in epithelial and inflammatory cells. Immunoreactivity of TLR2 appeared as brown cytoplasmic staining of varying degrees of intensity in epithelial cells and inflammatory cells. For negative control, the primary antibody was replaced by