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Histopathological and Histochemical Studies of Gut of Mouse During *Schistosoma mansoni* and *Schistosoma margrebowiei* Infections

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Abstract: Comparative histopathological and histochemical studies on the gut damage following infections with *Schistosoma mansoni* and *Schistosoma margrebowiei* were showed after 42, 49, 77, 91, 105 and 119 days post infection (d.p.i) in definitive host mouse. The prominent histopathological changes were thinning of epithelial cells and variable shape of the goblet cells. Most of the villi were shrunken, crackling and tips were damaged. Increases spaces and infiltration of the granulocytes were observed in lamina propria of the tunica mucosa. The granulomas with single or multiple eggs were mainly surrounded by variety of connective tissues observed in the submucosa, tunica muscularis and tunica serosa from 42 to 119 d.p.i. in *S. mansoni* and in *S. margrebowiei* from 77 to 119 d.p.i. *S. mansoni* eggs produced a minimum cellular reaction in the lamina propria of tunica mucosa from 91 to 119 d.p.i. A few eggs were seen in the blood vessels of the submucosa from 42 to 119 d.p.i. in *S. mansoni*. Due to infections swollen walls with enlarged endothelial cells of the blood vessels were observed. Fibrosis was seen in the tunica muscularis and tunica serosa from 91 to 119 d.p.i. A few areas of early necrosis were also observed in the tunica submucosa and tunica muscularis from 91 to 119 d.p.i. in both parasite species. Normal and infected brush borders, epithelial cells, goblet cells, muscular cells, connective tissues in the lamina propria of tunica mucosa and submucosa were positive for protein. The most prominent changes were increase of protein in paneth cells present in the crypts of gut during infections. Significantly increased amount of glycogen and acid mucopolysaccharides was observed in the goblet cells. Thin granules of glycogen were positively stained in the brush borders and also muscular cells of the tunica muscularis. Ribonucleic acid was stained positively in epithelial cells, connective tissues and muscular cells. Endothelial cells were stained weekly for ferric irons and lipofuscin pigments.

Key words: Histopathology, histochemistry, gut, mouse, *Schistosoma mansoni*, *Schistosoma margrebowiei*, schistosomiasis

INTRODUCTION

Schistosomiasis is caused by parasitic trematode worms (schistosomes) that reside in the abdominal veins of their vertebrate definitive hosts (Allen *et al*., 2002). Acute schistosomiasis (Katayama fever) (Sasa, 1972) is common in areas of high transmission rates. A history of contact with contaminated water 14 to 84 days before presentation is usual. Symptoms are thought to be mediated by the immune complex and the majority of cases begin with the deposition of an egg into host tissues (Doherty *et al*., 1996; Bethlem *et al*., 1997; Cooke *et al*., 1999). The disease is caused by hypersensitivity reaction against parasite eggs trapped in the venules. Eggs release antigens that produce varying degrees of granulomatous response in the intestines of the definitive host (Hirata *et al*., 1993). In the intestine eggs can provoke a patchy inflammatory infiltrate admixed with granulomas (Cheever *et al*., 1980). Granulomatous tissue causes loss of elasticity in the intestinal wall and as the disease progresses the tissue can become calcified (Warren, 1982). Eggs retained in the gut wall induce inflammation, hyperplasia, ulceration, micro-abscess formation and polyposis (Chen *et al*., 1978; Cheever and Duvall, 1982; Chen, 1991). Colicky hypogastric pain or pain in the left iliac fossa is frequent. Diarrhoea is common and may alternate with constipation. Diarrhoea is particularly common in children and its presence correlates strongly with schistosomiasis (Zhou *et al*., 1998). Occult (or sometimes visible) blood in the feces is usual. Severe chronic intestinal disease may result in colonic or rectal stenosis. Colonic polyposis may be manifested as a protein-losing enteropathy (Hussein *et al*., 1983). Inflammatory masses in the colon may even mimic cancer (Poon and Chu, 1999). The relation between colorectal cancer and schistosomiasis has been

debated for decades. If there is an increase in the risk of colorectal cancer, it is small (Chen and Mott, 1989; Yu *et al*., 1991; Ojo *et al*., 1992). The above findings reveal that the schistosome egg is the parasite factor responsible for schistosomal disease, but that the host granulomatous reaction to the eggs is a major factor in the pathogenesis of schistosomiasis (James and Colly, 1976). From above literature it is clear that few histopathological studies have been already done on the gut of mouse following infections with *S. mansoni*. But there is very less information available on the histochemical changes during schistosomiasis; however, this study describes both changes in acute and chronic *S. mansoni* and *S. margrebowiei* infections.

MATERIALS AND METHODS

Age-matched mice of the Bantam and Kingman Tyler's Original (BKTO) strain, weighed approximately 20-35 g each were infected with 200 and 25 cercariae of either *S. mansoni* (Puerto Rican strain) maintained in albino *Biomphalaria glabrata* snails and *S. margrebowiei* (originally obtained from Lochinvar National Park, Zambia) and maintained in *Bulinus natalensis* intermediate host snails (the original stock was obtained from the Experimental Taxonomy Unit of the British Museum of National History, London). Before administering the cercariae, experimental animals were anaesthetized with Sodium Pentobarbitone (Nembutal) and abdominal hairs were clipped. The cercariae were applied to the abdominal skin by using ring. All the mice infected with 200 cercariae were killed on day 42, while rests were killed at days 49, 77, 91, 105 and 119 d.p.i. Autopsies were performed immediately after the animals were killed by dislocation of neck region. The gut from each animal was fixed in Heidenhain's Susa fixative, washed and dehydrated in ethanol, infiltrated and embedded in historesin (Soomro, 1996). Selected 4 μm thick sections were stained in Polychrome method for general histology and variety of histochemical methods; including bromophenol blue, periodic acid mucopolysaccharide, ribonucleic acid, ferric iron and lipofuscin pigment demonstrations. This study was conducted in School of Biological Sciences, University of North Wales Bangor Gwynedd LL57 2 UW United Kingdom in 1998.

RESULTS

Histopathological changes in the *S. mansoni* and *S. margrebowiei* infected gut of mice: The granulomas with single or multiple eggs were mainly surrounded by variety of connective tissues. These including

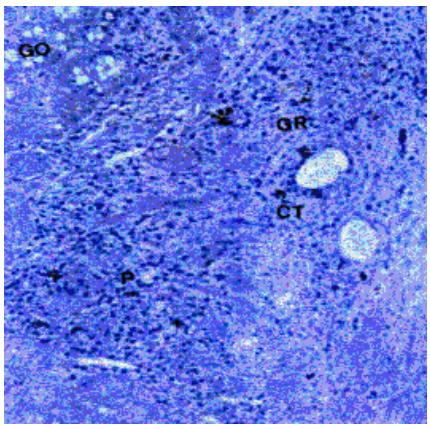

Fig. 1: After 91 dpi with *S. Mansoni* granuloma with double eggs, goblet, epithelial cells, connective and muscular tissues were staining weakly for protein

eosinophils, macrophages, lymphocytes, monocytes, plasma cells, polymorphonuclear cells and mast cells were observed in the submucosa, tunica muscularis and tunica serosa from 42 to 119 in *S. mansoni* (Fig. 1) and in *S. margrebowiei* from 77 to 119 d.p.i. Fibrosis with fibroblasts and mast cells were numerous in the tunica muscularis and tunica serosa from 91 to 119 d.p.i. A few areas of early necrosis were observed in the tunica submucosa and tunica muscularis from 91 to 119 d.p.i. in both species.

S. mansoni eggs were observed in the lamina propria of tunica mucosa from 91 to 119 d.p.i. and produced a minimum cellular reaction. The epithelial cells in the villi became thin and the shape of the goblet cells was variable. Most of the villi were shrunken, crackling and tips were damaged. Increases spaces and infiltration of the granulocytes were observed in the lamina propria of the tunica mucosa. A few eggs were also seen in the blood vessels of the submucosa from 42 to 119 d.p.i. in *S. mansoni*. Swollen walls with enlarged endothelial cells of the blood vessels were observed from day 42 to 119 d.p.i.

From the appearance of the control and infected gut of mice it seems that there were some changes in the size and shape of the following structures. The mean diameter (± standard deviation. of the uninfected goblet cells were 6.76±0.94 μm and of infected ones were 15.61±1.75 μm. The mean diameter (± standard deviation) of the uninfected lamina propria was 30.937±7.803 μm and of infected was 46.55±17.361 μm. The mean diameter (± standard deviation) of the uninfected crypts was 36.27±8.71 μm and around granulomas was 36.37±8.17 μm.

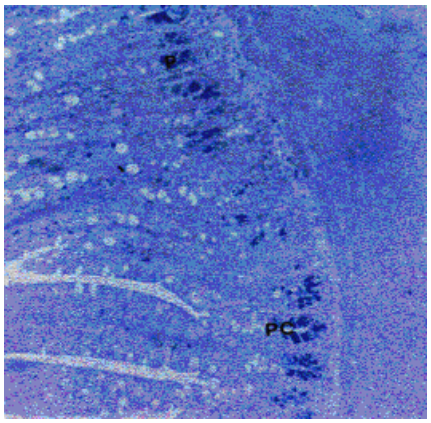

Fig. 2: After 105 dpi with *S. Mansoni* increased amount of protein was observed in paneth cells. Stain: Bromophenol blue

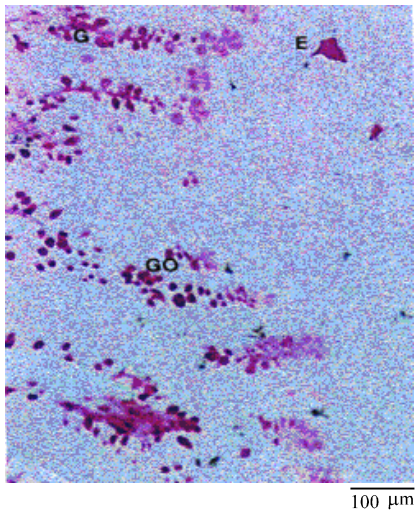

Fig. 3: After 91 dpi with *S. Mansoni* increased amount of glycogen was observed in goblet cells. Note that single egg is prominent in sub mucosa layer of the gut. Stain: Periodic acid schiff reaction

Histochemical changes in the normal *S. mansoni* and *S. margrebowiei* infected gut of mice: Normal and infected brush borders, epithelial cells, goblet cells, connective and muscular tissues of the gut were stained weakly for protein. Normal and abnormal dividing cells were stained mildly for protein (Fig. 1). Increased amount of protein was seen in paneth cells present in the crypts during infections (Fig. 2). Normal and infected nuclei of the connective tissues of the lamina propria and submucosa were stained positively for protein. Normal and abnormal nuclei of the endothelial vessels in the blood vessels in the submucosa were stained positively

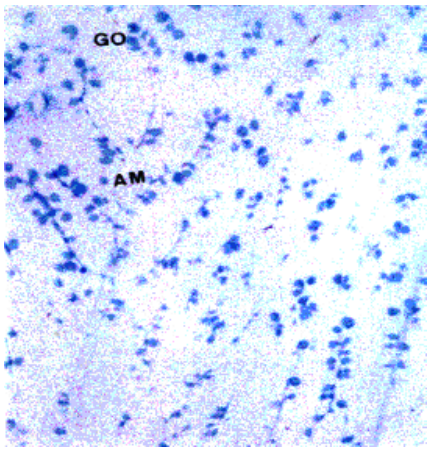

Fig. 4: After 91 dpi with *S. Monsoni* goblet cells were stained prominently for acid mucopplysaccahride, Stain: Acid mucopplysaccahride,

for protein. Normal and infected circular and longitudinal muscular layers of the tunica muscularis were stained positively for protein. Normal and infected brush borders stained positively for glycogen. In normal goblet cells glycogen was stained mildly but during both parasite infections significantly increased amount of this substance was seen (Fig. 3). Weakly stained granules of glycogen were observed in the lamina propria, submucosa and positively in the tunica muscularis of normal and infected gut of mice.

Normal and abnormal brush borders stained weakly for acid mucopolysaccharides. Normal goblet cells were stained mildly but in the infected gut of the mice there was an increased amount of acid mucopolysaccharides in both parasite infections (Fig. 4).

Weakly stained brush borders, nuclei and cytoplasm of the epithelial cells were positive and weakly for ribonucleic acids. Normal infected goblet cells and endothelial cells were positive for ribonucleic acids. Normal endothelial cells stained weakly around granulomas where these cells stained positively for ferric irons. Normal and abnormal endothelial cells stained weakly for lipofuscin pigments. Mast cells around the granulomas and fibrosis in the gut were stained positive for protein, glycogen, acid mucopolysaccharide, ribonucleic acids, weakly for ferric irons and negative for lipofuscin pigments.

DISCUSSION

In the present study some histopathological changes have been reported in normal, infected *S. mansoni* and *S. margrebowiei* infected gut of mouse. Due to the presence of single or multiple eggs thin epithelial cells, increased spaces and infiltration of the granulocytes were

seen in the lamina propria of the tunica mucosa. However, increased connective tissues reaction was noticed around the granulomas. Von Lichtenberg *et al.* (1971) and Cheever *et al.* (1980) have reported that in the intestine eggs can provoke a patchy inflammatory infiltrate admixed with granulomas. In the present study some granulomas were observed in the submucosa, tunica muscularis and tunica serosa in *S. mansoni* infection. The villi became thin, shrunken, crackling and tips were damaged. Warren (1982) has reported that granulomatous tissue causes loss of elasticity in the intestinal wall and as the disease progresses can become calcified. In the present study variety of connective tissue cells were observed around the granuloma. These findings are also in agreement with Andrade and Barka (1962). They have reported cells around granuloma probably correspond to basophilic reticular cells and are related to the gamma globulin containing cells.

In the present study fibrosis were seen in the submucosa, tunica muscularis and tunica serosa from 91 to 119 d.p.i. in *S. mansoni* infection. However, between 80 and 93 days after *S. mansoni* infection there was an increase in the intensity of the fibrotic reactions around eggs in the subserosa, muscular layer and these levels seen in the submucosa. These findings are nearly in agreement with those reported by Kloetzel (1970).

Some histochemical tests have been carried out on normal, infected *S. mansoni* and *S. margrebowiei* infected gut of mouse. In the present study granules of paneth cells were stained with bromophenol blue method for protein during *S. mansoni* and *S. margrebowiei* infections. Spicer *et al.* (1967) reported that such granules of paneth cells were stained with acid dyes, such as eosin or orange G. In the mouse, the paneth cell granules contain a sulfated mucosaccharide and a basic protein. The latter is believed to be lysozyme, an enzyme found in tears, leukocytes and mucous secretion, which lysis bacteria. The presence of this enzyme suggests an antibacterial function of paneth cell secretion.

In the present study using light microscope on the histochemistry of the intestine of the BKTO mouse increased amount of glycogen was seen in the goblet cells during *S. mansoni* and *S. margrebowiei* infections. Whereas, an other study using the electron microscope on the cytochemistry of the intestinal epithelium of C3H mice, showed glycogen masses along with lipid droplets of various sizes during *Echinostoma caproni* infection (Fujino *et al.*, 1993).

In the present study acid mucopolysaccharides and Periodic acid and Schiff reaction, the majority of goblet cells were blue and pink respectively. The goblet cells were seen in the villi. However, Brown *et al.* (1988) have reported in alcian blue/periodic acid Schiff reaction (AB/PAS) stained sections majority of the goblet cells were stained shades of the purple.

In the present study variable size and shapes of goblet cells stained with PAS positive were seen in the villi or crypts of gut of mouse. These results are in agreement with the findings of the Brown *et al.* (1988). They reported that the goblet cells of upper and lower small intestine white pigs stained with PAS alone occurred occasionally at all levels of the crypts or villus. Fujino *et al.* (1996) have reported that in controlled mice infected with *E. trivovis* goblet cells were stained heavily with AB-PAS and showed a marked increase in number. In the present study goblet cells of schistosome infected mice were also stained heavily with these staining methods.

The intestinal mucosa appears to be selective in the absorption of iron in its own right (Finch, 1950). The iron taken into mucosal cells is united with protein called apo-ferrotin to form ferritin. When the mucosal cells become saturated with ferritin no more iron is absorbed; this is referred to as the mucosal block hypothesis. Ferritin in the mucosal cells is in equilibrium with iron store of the body and iron in storage falls below a critical level more iron is permitted to pass the intestinal mucosa. The mucosal block hypothesis has not with stood experimental challenge and, therefore, the search continues for the explanation of iron transport cross the intestinal mucosa (Brown *et al.*, 1958; Chodos *et al.*, 1957). In the present study no any ferric iron was seen in the intestinal mucosa of the gut of mouse during both parasite infections.

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