

## HISTOCHEMICAL STUDIES OF SPLEEN OF MOUSE DURING SCHISTOSOMIASIS

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### ABSTRACT

Comparative histochemical studies on the spleen damage following infections with *Schistosoma mansoni* and *Schistosoma margrebowiei* have been reported after 42, 49, 77, 91, 105 and 119 days post-infection (p.i.) in definitive host mouse. The most prominent changes were the protein stained positively in capsule and trabeculae with mild amounts observed in red and white pulps of the spleen of mouse. Glycogen was stained weakly in capsule and white pulp, however, an increased amount was observed in red pulp of the spleen from 42 to 119 days p.i.. *S. mansoni* and *S. margrebowiei* egg embryos were stained markedly for glycogen from 91 to 119 days p.i.. Acid mucopolysaccharide was stained weakly in the spleen of mouse. Increased amount of ribonucleic acid was observed in nuclei of connective tissue, endothelial cells and megakaryocytes present in the spleen. Mast cells around the granulomas and fibrosis were stained positively for protein, glycogen, acid mucopolysaccharide, ribonucleic acid, weakly for ferric irons and negatively for lipofuscin pigments from 91 to 119 days p.i.. Ferric iron was absent from normal capsule and white pulp, but small amounts were present in trabeculae and red pulp of the spleen in both parasite infections. The endothelial cells in the blood vessels walls were stained positively for lipofuscin pigments in normal and after all parasite infections.

### INTRODUCTION

Splenomegaly is characteristic of schistosome infections, and is probably mainly due to increased portal blood pressure, venous stasis and also it seems due to antigenic stimulation causing lymphoreticular hyperplasia (Dumont *et al.*, 1975). The spleen in many cases of Symmer's fibrosis increases in size due to congestion caused by portal hypertension and hyperplasia of reticulo-endothelial elements (Andrade and Abreu, 1971). The major factor in the pathogenesis of schistosomiasis is the host's granulomatous response to eggs trapped in the tissues (Von Lichtenberg, 1962) and increased connective tissue reaction in the portal spaces (Fisher-Rostock, 1930; Belding, 1942; Craig and Faust, 1949; Ito, 1954). Homogenization of eggs in saline yields a supernatant containing soluble egg antigens (SEA). These are capable of pre-sensitising animals to give enhanced responses to injected whole eggs or egg products (Von Lichtenberg, 1962). Few histopathological studies have been done on the spleen of mouse following infection with *S. mansoni*. However, this paper describes the histochemical changes in acute and chronic *S. mansoni* and *S. margrebowiei* infections.

### MATERIALS AND METHODS

Age-matched mice of the Bantim and Kingman Tylers Original (BKTO) strain, weighing approximately 20-35g each were infected with 200 and

25 cercariae of either *S. mansoni* (Puerto Rican strain) maintained in albino *Biomphalaria glabrata* snails and random-bred TO mice method of Taylor *et al.*, (1969) and *S. margrebowiei* (originally obtained from Lochinvar National Park, Zambia) maintained in *Bulinus natalensis* intermediate host snails (the original stock was obtained from the Experimental Taxonomy Unit of the British Museum of Natural History, London). Before applying the cercariae, the experimental mice were anaesthetised with Sodium pentobarbitone (Nembutal) and the abdominal hairs were clipped. The cercariae were applied to the abdominal skin by using ring. The mice infected with 200 cercariae were killed on day 42; while mice infected with 25 cercariae were killed at days 49, 77, 91, 105 and 119 p.i.. Autopsies were performed immediately after the mice were killed by dislocation of neck region. The spleen from each animal were 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 with different histochemical methods; bromophenol blue for protein, Periodic acid and Schiff reaction for glycogen, alcian blue for acid mucopolysaccharide, ribonucleic acid for RNA, ferric iron and lipofuscin pigment.

### RESULTS

The protein was present positively in the cells of connective tissue and muscle of capsule and trabeculae with mild amounts observed in red and white pulps of the spleen in both parasite infections. The capsule and

white pulp were stained weakly, however, an increased amount of glycogen was observed in red pulp of spleen from day 42 to 119 p.i.. *S. mansoni* and *S. margrebowiei* egg embryos were stained markedly for this substance from 91 to 119 days p.i.. Acid mucopolysaccharide was stained weakly in areas of capsule and red pulp of the spleen in both parasite infections. From 42 to 119 days p.i., ribonucleic acid was stained mildly in the nuclei of connective tissues, muscle cells, endothelial cells and megakaryocyte present in the spleen, however, this substance was reduced in their cytoplasm. Ferric iron was absent from normal capsule and white pulp, but small amount was present in trabeculae and red pulp of the spleen in both parasite infections. The endothelial cells in the blood vessels walls were stained positively for lipofuscin pigments in normal and after all parasite infections. Mast cells appeared around the granulomas and fibrosis in the spleen were stained positively for protein, glycogen, acid mucopolysaccharide, ribonucleic acid, weakly for ferric iron and negatively for lipofuscin pigments from 91 to 119 days p.i..

### DISCUSSION

Histochemical changes in the *S. mansoni* and *S. margrebowiei* infected spleen of mouse has been reported for the demonstration of protein, glycogen, acid mucopolysaccharide, ribonucleic acid, ferric iron, and lipofuscin pigment. In the present study protein was stained positively in capsule and trabeculae, with mild amount observed in red and white pulps of the spleen of mouse in both parasite infections.

Glycogen was stained weakly in the capsule and white pulp, while increased amount of this substance was observed in red pulp of the spleen from 42 to 119 days p.i.. Acid mucopolysaccharide was stained weakly in the above areas of the spleen of mouse. Increased amount of the ribonucleic acid was stained in the nuclei of cells, but lower amount in the cytoplasm.

In present study ferric iron was absent from normal capsule and white pulp, but small amount was present in the trabeculae and red pulp of spleen in both parasite infections. Other observations reported by Schalm, (1965) that excess iron is stored as ferritin in the liver, spleen and bone marrow. When haemoglobin is broken down in the removal of over-aged or abnormal erythrocytes, the iron becomes immediately available for conversion into haemoglobin again. An excess of iron in the tissues produces haemochromatosis. Such excess iron is irritating and leads to development of cirrhosis in the liver, spleen

and pancreas. In present study the endothelial cells were stained positively for lipofuscin pigments during infections. This study will provides additional information on histochemical changes in the spleen of mouse during schistosomiasis.

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