Carbohydrates Detection in the Hepatic Egg - Granuloma System Using Lectin Histochemistry

Detección de Hidratos de Carbono en el Sistema Granuloma-Huevo Hepático Usando Lectina Histoquímica

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SUMMARY: This study aims to evaluate the egg-granuloma system in hepatic tissues using lectin histochemistry in experimental Schistosomiasis. Eight Swiss mice were infected with a local strain of *Schistosoma mansoni*, being submitted forty days later to a perfusion after which slices of liver (4µm) were prepared. The tissue samples were incubated with the following peroxidase conjugated lectins: Peanut agglutinin (PNA), Wheat Germ agglutinin (WGA), and Concanavalin A (Con A). All lectins recognized the glycoconjugates in the adult worm tegument. In the hepatic tissue, WGA presented the highest staining followed by PNA and Con A. The PNA presented the most intense staining of the egg-granuloma system while WGA stained the hepatic sinusoid cells and Con A bound preferentially the fibrosis rings of granuloma and the surrounding hepatic parenquima. WGA and PNA indicated the presence of residues of N-acetyl-glucosamine and galactose in the surface of *Schistosoma mansoni* eggs in the hepatic granulomas. In conclusion, using PNA, Con A and WGA our study presented different aspects of the egg-granuloma and Tegument of *Schistosoma mansoni* as well as indicated differences in the peri-ovular granulomas indicating alterations in the cellular mechanism of expression of surface carbohydrates during progression of the Schistosomiasis.

KEY WORDS: Lectins; Egg-granuloma system; Liver; *Schistosoma mansoni*.

INTRODUCTION

Lectins, carbohydrate-binding ubiquitous proteins, have found numerous applications in biological and medical areas due to their broad recognition properties (Lehmann *et al.*, 2006). They have been used as probes for prognosis and/or diagnosis in human and animal diseases (Mitchell & Schumacher, 1999). In histochemistry, lectins with different carbohydrate specificity can provide a sensitive detection system for changes in glycosylation and carbohydrate expression that may occur during embryogenesis, growth and disease and are sensitive, stable and easy-to-use tools to distinguish transformed from non-transformed cells (Ji *et al.*, 2004).

The lectin-carbohydrate interactions have been used to survey the development of parasites since their antigenic glycoconjugates are responsible for immune response in animal infections (Hundt *et al.*, 2000). *Schistosoma mansoni* antigens have been investigated using lectin histochemistry (Schottelius & Aisien, 1994; Okano *et al.*, 1999; van Vliet *et al.*, 2005). In different stages of development cell

membrane glycoconjugates of *Schistosoma* have been found to be associated to the immune response level after infection (Nyame *et al.*, 2000).

The tegument of *Schistosoma* eggs have complex structure where the cytoskeleton is rich in carbohydrates with extreme dynamic features that are used as defence barrier to the host immune response as well as to drug action (van Vliet *et al.*).

Recent researches have characterized the surface carbohydrates of parasites during infection development (Restrepo *et al.*, 2000; Hokke & Yazdanbakhsh, 2005). Mapping of sugar residues of the surface of *S. mansoni* egg allowed identifying the strain of the infectious parasite and the host immune defence response against it, although many patho-biochemical changes in the cellular environment of the liver and intestine during infection by *S. mansoni* remain unknown (Muller *et al.*, 2001).

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Since little attention has been given to the physiology of the egg-granuloma system, this study aims to evaluate this system in hepatic tissues using lectin histochemistry in experimental schistosomiasis developed from a local strain of *S. mansoni* (SLM, Pernambuco - Brazil) isolated from *Biomphalaria glabrata*.

MATERIAL AND METHOD

Experimental Model. Eight Swiss mice were infected with a local strain of *S. mansoni* (SLM from São Lourenço da Mata – Zona da Mata of the State of Pernambuco - Brazil) isolated from *B. glabrata*. Infection was performed through tail immersion for 2h into an aqueous solution containing 50 cercariae. After forty days, the mice were submitted to perfusion and livers were preserved in 10% buffered-formalin solution until the inclusion of paraffin. Slices (4µm) were prepared and haematoxylin and eosin staining was developed.

Lectin Histochemistry. Concanavalin A (Con A), Wheat germ agglutinin (WGA) and Peanut agglutinin (PNA) lectins conjugated to peroxidase were supplied by Sigma Chemical Company (USA). Paraffin sections (4 µm) were deparaffinized in xylene and hydrated through graded alcohol. Tissue sections were treated with 0.1% (w/v) trypsin solution, at 37°C for 2 min, followed by 0.3% (v/v) methanolic hydrogen peroxide, for 20 min. and incubated with conjugated lectins (Con A, 25 µg/mL; WGA and PNA, 50 µg/mL), for 2h, at 4°C. Slides were washed (two 5-min) with 10 mM phosphate buffer, containing 0.15 M NaCl (PBS), pH 7.2, after each step. Peroxidase was visualized by incubation for 5 to 8 min in PBS containing diaminobenzidine (DAB) and hydrogen peroxide. The tissue sections were counterstained with haematoxylin and evaluated through optical microscopy. Inhibition assays were performed using the corresponding lectin specific sugar: methyl- α -D-mannoside for Con A, N-acetyl-glucosamine for WGA and D-galactose for PNA, at final concentration of 300 mM.

Morphometric analysis. Images of histological slices were captured using a digital video camera (Sony-USA) connected to a microscope and were processed using the OptimasTM 6.1 (Optimas Corporation, USA) image analysis programme. Lectin-binding patterns were used to calculate the mean area of (mm²) and the mean number of cells (weak stain = 15-20%; moderate stain = 25-55%; intense stain = 60-95%). Semi-quantitative optical analysis was carried out taking account the intensity of staining pattern indicated as weak (+), moderate (++) or intense (+++), according to Özer (2000).

RESULTS

In this study the lectin histochemistry was used to evaluate the egg-granuloma system in experimental schistosomiasis. All used lectins recognized the glycoconjugates in the adult worm tegument (AWT) in the hepatic tissue where WGA presented the highest staining followed by PNA and Con A.

WGA recognized carbohydrates residues in the parasites surface in a homogenous pattern presenting an intense staining at the hepatic sinusoid vessels, as well, during the chronic phase of the disease (Fig. 1).

Fig. 1. N-acetyl-glycosamine carbohydrate in the egg-granuloma system and granuloma fibrosis rings recognized by stained by WGA. 100x.

The egg-granuloma system was uniform and intensively stained by PNA, being this lectin the best one to characterize disease features. PNA presented also a positive staining to the AWT (Fig. 2).

Fig. 2. Galactosis carbohydrate into adult worm tegument of *Schistosoma mansoni* indicated by PNA binding. 100x.

Con A recognized preferentially the fibrosis rings of the granulomas and their adjacent hepatic parenquima. Con A presented the most unspecific binding pattern among the three lectins used. WGA and PNA indicated the presence of N-acetyl-glucosamine and galactose, respectively, in the surface of *S. mansoni* eggs in the hepatic granuloma environment (Table I).

Table I. Histological reactivity of the lectins (staining pattern) into hepatic tissues infected by *Schistosoma mansoni*.

Structure	Lectin*		
	WGA†	PNA	Con A
Adult worm tegument	+++	+++	+++
Egg-granuloma	-	++	+
Hepatic tissue	-	-	+

Staining pattern: none (-); weak (+); Moderate (++); Intense (+++).

WGA=Wheat germ agglutinin; PNA=Peanut agglutinin; Con A=Concanavalin A.

Lectin binding was completely abolished when the lectins were previously incubated with their respective carbohydrate at final concentration of 300 mM. This result confirms the staining pattern observed after lectin histochemistry of the schistosomiasis experimental model.

DISCUSSION

Lectin carbohydrate recognition ability has been used to explore different aspects of saccharide content and distribution on cell surface or tissues (Haseley *et al.*, 1994). *Schistosoma mansoni* have been studied in the Brazil for almost hundred years. Epidemiology and histopathology are among the most studied aspects of schistosomiasis since then (Rutitzky *et al.*, 2005).

Molecular, immunohistochemical and histochemical techniques are used to investigate the development of *Schistosoma* eggs and the role of their secretions in the initiation, development and activation of immune response in the host tissues (Ashton *et al.*, 2001; Lehmann *et al.*).

The distinct localization of the staining patterns observed in our study using Con A, WGA and PNA, which have different carbohydrate specificity, indicated the expression of certain sort of saccharides in the egg-granuloma system developed experimentally.

Experimental evidences suggest that sugars of the cell surface are involved in the processes of recognition

between parasite and host and that changes in this array of surface carbohydrates induce structural and functional modifications in the host cells (Hartgers, 2006).

Here, positive staining was observed to *S. mansoni* parasite tegument for the used lectins, although PNA, Con A and WGA failed to distinguish between the female and male parasites via carbohydrate array present in their tegument as reported by Gobert *et al.* (1998) for *S. japonicum* and *S. mansoni*. Similar results were found with other lectins (PHA and UEA-I) used for the characterization of the AWT of *S. japonicum* (Gazzinelli *et al.*, 1991).

The interest in mapping the glycoconjugates in *S. mansoni* tegument arose from the fact that during its development, the worm incorporates in its membrane glycolipids, glycoproteins and immunoglobulins from the host. Since these structures are not recognized as non-self by the host, the worms become safe from the attack of the host antibodies (Faveeuw, 2002).

Our results showed that the egg-granuloma system was evidenced by the PNA and Con A binding. One possible explanation for this lectin binding pattern is that the lectins recognized glycoconjugates present in the egg surface (Jacobson & Doyle, 1996) or egg secreted glycoproteins diffused through and anchored in the adjacent granulomas tissue characterizing glycoconjugates expression by the granuloma cells (Ashton *et al.*).

In this work, PNA binding pattern was diffuse and non-specific for the cellular environment present in the granulomas. Studies have observed that carbohydrates of eosinophils surface are involved in the degranulation process (Ohmori & Nawa, 2000), fact which raised attention for the importance of expression and liberation of products from cells in the hepatic granulomas acting as fibrosis inhibitors (van de Vijver *et al.*, 2006).

Lectin binding intensity can be influenced by the stage of development of the *Schistosoma* egg, reflecting the content of carbohydrates in the egg tegument. Ashton and co-workers observed that mature eggs present a higher quantity of (glyco) proteins than the former egg stage. This difference was observed in our study since Con A presented a different binding pattern to different stages of the egg-granuloma system. D-glucose and D-mannose were previously indicated as sugars involved in the parasite-host interaction during infection (Jacobson & Doyle; Lee *et al.*, 1999; Opanasopit *et al.*, 2001).

Recently, many studies involved were developed in our laboratories to investigate different pathologic

process (Melo-Junior *et al.*, 2004; Melo-Junior *et al.*, 2006a; 2006b; Araújo-Filho *et al.*, 2006) indicating an important contribution of lectins in those biomedical analysis.

Lectin histochemistry has shown to be an interesting tool in the investigation of the structure and function of glycoconjugates in the parasites and host-cells besides helping to light the biochemical alterations during the evolution of parasitosis (Nyame *et al.*; Eichinger, 2001).

In conclusion, using PNA, Con A and WGA our study presented different aspects of the egg-granuloma and

AWT of *S. mansoni*, as well as indicated differences in the peri-ovular granulomas showing alterations in the cellular mechanism of expression of surface carbohydrates during the progression of schistosomiasis.

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RESUMEN: El objetivo del estudio fue evaluar el sistema de los huevos de los granulomas en los tejidos hepáticos, utilizando histoquímica de lectinas esquistosomiasis. Ocho ratones suizos experimentales fueron infectados con una cepa local de *Schistosoma mansoni* y luego a los cuarenta días fueron sometidos a la perfusión y se prepararon cortes de hígado (4 µm). Las muestras de los tejidos fueron incubadas con las siguientes peroxidadas lectinas conjugadas: aglutinina de maní (PNA), aglutinina de germen de trigo (WGA), Concanavalin A (Con A). Todas las lectinas reconocieron las glicoconjugadas en el tegumento del gusano adulto. El tejido hepático con WGA presentó mayor coloración seguida de PNA y Con A. El PNA presentó la más intensa tinción de los huevos mientras el granuloma del sistema WGA tiñó las células hepáticas sinusoides y las Con A estuvieron siempre presentes en los anillos de la fibrosis y alrededor de los granulomas hepáticos del parénquima. WGA y PNA indicaron la presencia de residuos de N - acetil - glucosamina y galactosa en la superficie de los huevos de *Schistosoma mansoni* en los granulomas hepáticos de esquistosomiasis.

PALABRAS CLAVE: Lectinas; Sistema huevo-granuloma; Hígado; *Schistosoma mansoni*.

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