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Lectin Receptors in the Gut Epithelium of *Schistosoma japonicum* and *Paragonimus ohirai*

TAKAHIRO FUJINO[1], YOICHI ISHII[1] AND MIZUKI HIRATA[2]

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Abstract

Glycoconjugates were localized in the gut epithelium of adult *Schistosoma japonicum* and juvenile and adult *Paragonimus ohirai* by a postembedding method using a variety of lectin-colloidal gold probes prepared with concanavarin A (Con A) and agglutinins derived from *Dolichos biflorus* (DBA), *Limax flavus* (LFA), *Arachis hypogaea* (PNA), *Ricinus communis* (RCA-I), soybean (SBA), *Ulex europaeus* (UEA-I) and wheat germ (WGA). Colloidal gold probes prepared with WGA, RCA-I and SBA bound selectively to N-acetylglucosamine, D-galactose and N-acetylgalactosamine residues, respectively, in the gut epithelium of both species. SBA binding was weak. The patterns and intensity of binding of these lectins differed to some extent between species and between developmental stages of *P. ohirai*. Labelling occurred in Golgi vesicles and on the surface coat of cytoplasmic projections in the gut of *S. japonicum*. By contrast, labelling occurred on cytoplasmic secretory granules and on fine linear structures between lamellar cytoplasmic projections of the gut of *P. ohirai*. Specificity of binding was controlled by competitive inhibition with specific sugars.

Key words: *Schistosoma japonicum, Paragonimus ohirai,* lectins, gut epithelium, ultrastructure

Introduction

Lectins, which bind to specific terminal carbohydrates of glycoconjugates, have been used widely as specific markers for the localization of carbohydrate components of the cell surface and intracellular structures. The ultrastructural localization of glycoconjugates in the tegument of some cestodes and nematodes has been accomplished with a lectin-gold labelling method (Schmidt and Peters, 1987; Schraermeyer *et al.*, 1987; Schmidt, 1988).

A gut-derived circulating antigen has been identified in the serum of laboratory animals and humans infected with *Schistosoma mansori* or *S. japonicum*. This antigen is a genus-specific proteoglycan composed primarily of N-acetylgalactosamine and D-glucuronic acid residues with minor amounts of amino acids (Nash *et al.*, 1974, 1977). Circulating antigens produced by adult parasites have been demonstrated in the gut epithelium by immunofluorescent techniques (von Lichtenberg *et al.*, 1974; Nash, 1974) and by immunocytochemistry or immuno-gold labelling procedures (Fujino *et al.*, 1985, 1988; de Water, 1986a, b). There have been no reports, however, on the detailed localization of carbohydrate residues in association with antigens or intracellular structures in the gut epithelium of trematodes. In this investigation, terminal sugar residues were localized in the gut epithelium of adult *Schistosoma japonicum* and juvenile and adult *Paragonimus ohirai* by a gold-labelled lectin technique.

Materials and Methods

Adult *Schistosoma japonicum* (50-day-old) were removed from the portal vein of experimentally infected mice (C57 BL). Juvenile (5-day-old) and adult (50-day-old) *Paragonimus ohirai* were recovered from the abdominal cavities and lungs, respectively, of albino rats (SD). After the

[1] *Department of Parasitology, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan*
[2] *Department of Parasitology, School of Medicine, Kurume University, Kurume 839, Japan*
藤野隆博　石井洋一（九州大学医学部寄生虫学教室）
平田瑞城（久留米大学医学部寄生虫学教室）

worms were washed in Ringer's saline, they were cut into small pieces and fixed for 90 min at 4°C with a mixture of 2% paraformaldehyde and 0.3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) containing 2% sucrose and 5×10^{-4}M $CaCl_2$. The tissues were dehydrated with an ethanol series, infiltrated overnight with LR White resin (London Resin Co. Ltd., Basingstoke, England), and then placed in gelatin capsules with fresh resin. The sealed capsules were polymerized at 55°C for 20 h. Ultrathin sections were cut and picked up on unsupported 400 mesh nickel grids.

Prior to labelling, the grids were floated on drops of 1% bovine serum albumin in phosphate buffered saline (PBS) for 20 min to block nonspecific labelling. The grids were washed in PBS and then incubated for 1 h at room temperature with Con A, DBA, LFA, PNA, RCA-I, SBA, UEA-I and WGA lectin-gold probes (15 nm diameter, E•Y Lab., Inc., San Mateo, CA.), that had been diluted 1/5 – 1/10 with PBS. Specificity of labelling was confirmed by mixing lectin-gold probes with their complementary sugars. After incubation with gold probes, the grids were washed with several changes of PBS and rinsed with distilled water. The grids were double stained with 1% uranyl acetate and lead citrate and examined with Hitachi HS-9 or H-500 electron microscope operated at 75 kV.

The intensity of gold-labelling was scored on a scale from 0 to 4+. The number of gold granules/0.5 μm^2 was counted on the surface of luminal cytoplasmic projections of the gut epithelium in electron micrographs.

Results

Our results showed a positivity in the gut epithelium of adult *Schistosoma japonicum* and juvenile and adult *Paragonimus ohirai* for WGA,

Table 1 Binding of lectin-gold to surface coat of cytoplasmic projections of gut epithelium

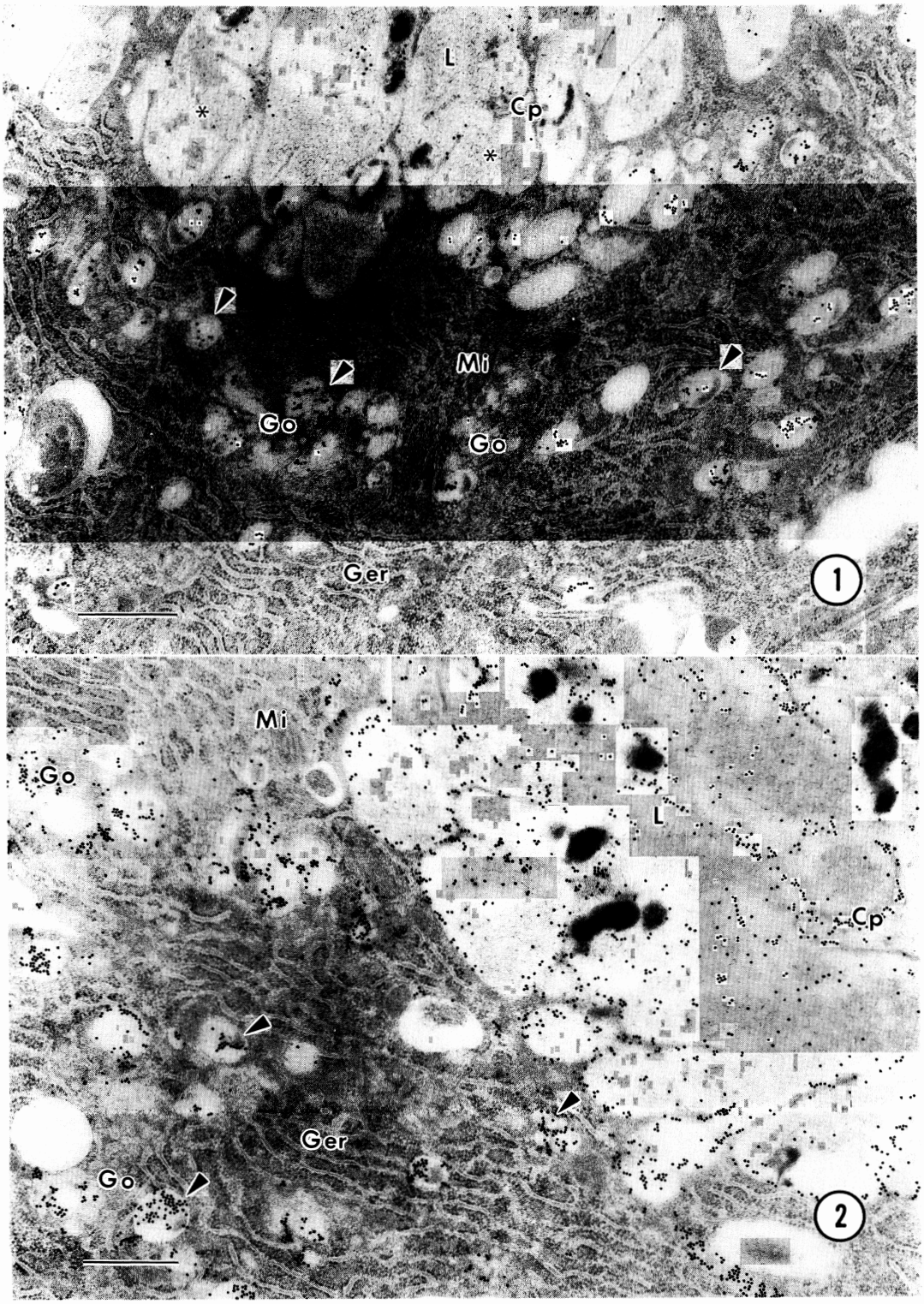
Lectin	Sugar specificity	Intensity of binding		
		S. japonicum	*P. ohirai*	
		Adult	Juvenile	Adult
WGA	GlcNAc, NeuNAc	2+	3+	2+
RCA-I	Gal	4+	2+	1+
SBA	GalNAc, Gal	1+/0	1+/0	1+/0
Con A	Man, Glc, GlcNAc	0	0	0
DBA	GalNAc	0	0	0
LFA	NeuNAc	0	0	0
PNA	Gal-GalNAc, Gal	0	0	0
UEA-I	α-L-Fuc	0	0	0

Number of gold particles per 0.5 μm^2; 4+, >50; 3+, 30–49; 2+, 15–29; 1+, 3–14; 0, 0–3

Abbreviations: GlcNAc, N-acetylglucosamine; NeuNAc, N-acetylneuraminic acid; Gal, Galactose; GalNAc, N-acetylgalactosamine; Man, Mannose; Fuc, Fucose

Fig. 1. *Schistosoma japonicum*. Gut epithelium of a 50-day-old adult labelled with WGA-gold. Labelling occurs in Golgi vesicles (arrowheads) and on the surface coat of cytoplasmic projections. Finely granular, moderately electron-dense material (*) is not labelled. Cp: Cytoplasmic projection; Ger: Granular endoplasmic reticulum; Go; Golgi complex; L: Lumen; Mi: Mitochondrion. Bar = 0.5 μm.

Fig. 2. *Schistosoma japonicum*. Gut epithelium of a 50-day-old adult labelled with RCA-I-gold. Gold particles are localized in Golgi vesicles (arrowheads) and on the surface coat of cytoplasmic projections. Some particles are in the lumen. Cp: Cytoplasmic projection; Ger: Granular endoplasmic reticulum; Go: Golgi complex; L: Lumen; Mi: Mitochondrion. Bar = 0.5 μm.


L
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RCA-I and SBA, whereas no reactivity for Con A, DBA, LFA, PNA and UEA-I was encountered. All observations with different lectins in cytoplasmic projections of the gut epithelium of both of the species are summarized in Table 1.

WGA labelling:

On sections of *S. japonicum,* labelling with WGA-gold occurred selectively in Golgi vesicles in the cytoplasm and in the surface coat of cytoplasmic projections (Fig. 1). Gold particles were also seen on the epithelial surface between the projections. Finely granular, moderately electron-dense amorphous material, which was partially enfolded by the projections, was not labelled.

On sections of juvenile and adult *P. ohirai,* gold particles were localized on fluffy material and fine linear structures on or between the lamellar cytoplasmic projections (Figs. 3 and 4). Amorphous material located in the gut lumen away from the epithelial surface was weakly labelled. Cytoplasmic secretory granules were also labelled. Marked differences were not observed in patterns of lectin binding between juveniles (Fig. 3) and adults (Fig. 4), although the intensity of labelling was stronger in juveniles (Table 1). Addition of 0.5 M N-acetylglucosamine to the incubation medium had little inhibitory effect on lectin binding. By contrast, addition of 10 mM triacetyl chitotriose effectively inhibited binding.

RCA-I labelling:

On sections of *S. japonicum,* intense labelling with RCA-I-gold occurred on or over cytoplasmic projections of the gut surface and on the epithelial surface between the projections (Fig. 2; Table 1). Amorphous material in the gut lumen was not labelled. Golgi vesicles were labelled, with small vesicles more intensely labelled than large ones.

On sections of *P. ohirai,* the distribution of gold label was similar to that of WGA, however, intensity of labelling was weaker. RCA-I-gold bound at a rather high density to amorphous material enclosed by cytoplasmic projections. Fewer numbers of gold particles were scattered on cytoplasmic projections and secretory granules. Incubation of sections with 0.3 M D-galactose completely inhibited the binding of RCA-I-gold.

SBA labelling:

On sections of *S. japonicum,* very weak labelling with SBA-gold was found over Golgi vesicles and the surface coat of cytoplasmic projections (Table 1).

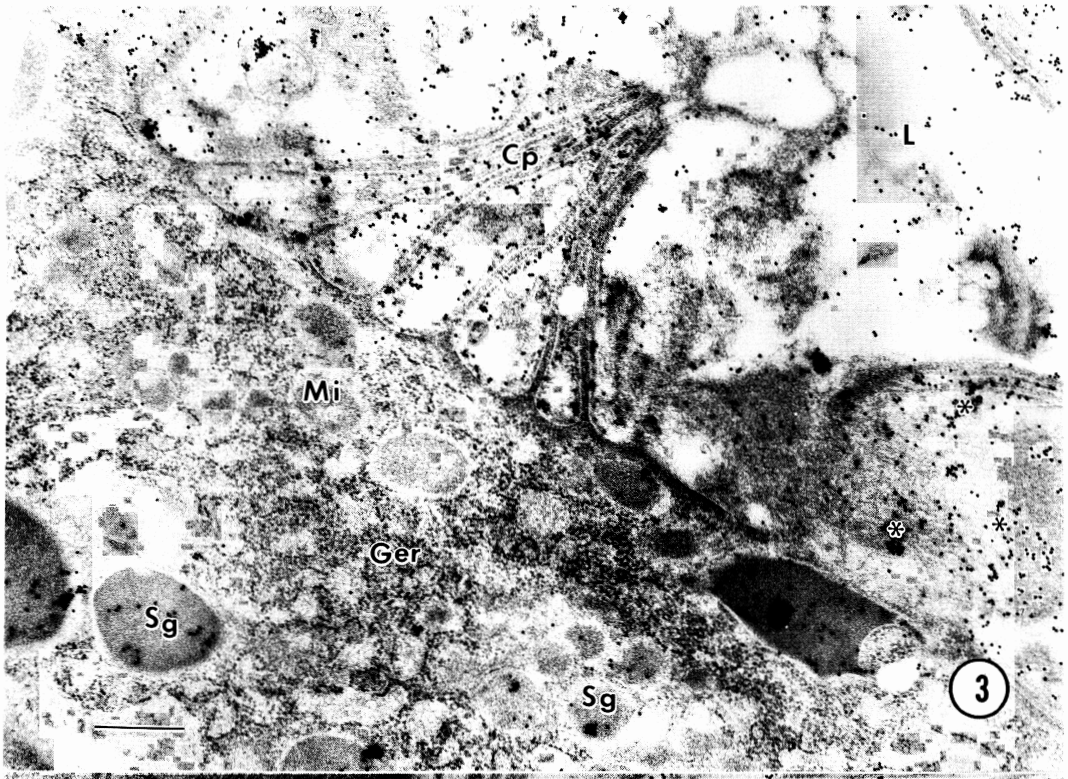
On sections of *P. ohirai,* weak labelling was found on the surface of cytoplasmic projections. Secretory granules were not labelled. Incubation of sections with 0.3 M N-acetylgalactosamine inhibited the binding of SBA-gold.

Discussion

Lectin binding sites were precisely localized in the gut epithelium of *Schistosoma japonicum* and *Paragonimus ohirai* with colloidal gold conjugates of WGA, RCA-I and SBA, indicating that N-acetylglucosamine, D-galactose and N-acetylgalactosamine are specific carbohydrate components of the gut epithelium. Some differences in binding patterns or intensity of gold labelling were detected between these two species and between juvenile and adult *P. ohirai* (Table 1). Binding of these lectins to either the surface coat of luminal cytoplasmic projections or to secretory granules and Golgi vesicles in the epithelial cytoplasm suggests that the molecules containing these sugar residues are synthesized on the granular endoplasmic reticulum, transported to the Golgi complex and eventually integrated

Fig. 3. *Paragonimus ohirai.* Gut epithelium of a 5-day-old juvenile labelled with WGA-gold. Amorphous material and rod-like, linear structures (*) on or between cytoplasmic projections are labelled. Secretory granules in the cytoplasm are also labelled. Cp: Cytoplasmic projection; Ger: Granular endoplasmic reticulum; L: Lumen; Mi: Mitochondrion; Sg: Secretory granule. Bar = 0.5 μm.

Fig. 4. *Paragonimus ohirai.* Gut epithelium of a 50-day-old adult labelled with WGA-gold. Labelling is similar to that of juvenile (Fig. 3), but with less intensity. Cp: Cytoplasmic projection; Ger: Granular endoplasmic reticulum; L: Lumen; Mi: Mitochondrion; Sg: Secretory granule. Bar = 0.5 μm.


Cp
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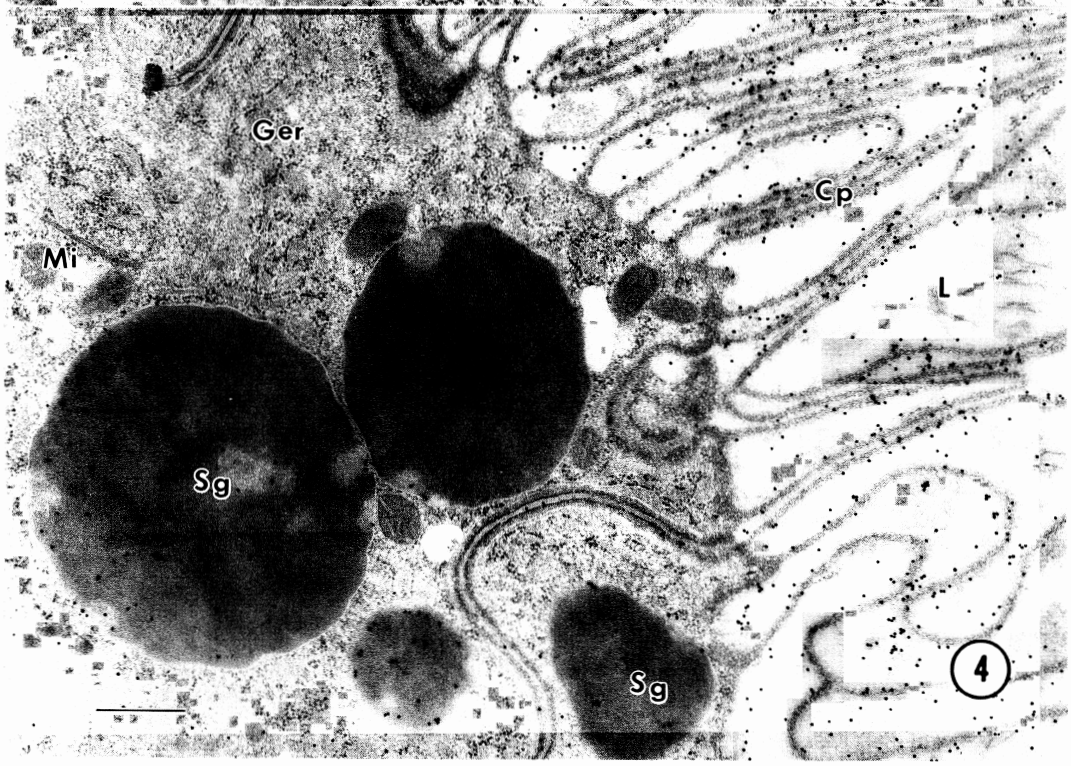
3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into secretory granules and exported to the gut lumen.

Carbohydrates may be targets for an immune response and it is important to characterize structural components of the surface of helminth parasites. Tegumental glycopeptides of *S. mansoni* have been studied with labelled lectins (Wilson and Barnes, 1977; Bennett and Seed, 1977; Murrell *et al.*, 1978; Simpson and Smithers, 1980; Linder and Huldt, 1982; Zelck and Becker, 1990). Linder and Huldt (1982) showed the distribution of exposed and hidden carbohydrates in the tegument, gut and eggs of *S. mansoni* with fluorochrome-conjugated lectins. A strong selective staining with SBA and RCA was seen in the gut epithelium, but no staining was observed with WGA. Binding of RCA to the gut epithelium of *S. japonicum* in the present study was similar to previous observations of *S. mansoni,* but reactivity of WGA and SBA was different.

Nash (1974) and von Lichtenberg *et al.* (1974) localized circulating antigen in adult *S. mansoni* with an indirect immunofluorescent technique. Fujino *et al.* (1985, 1988) and de Water *et al.* (1986a, b) demonstrated the ultrastructural localization of this antigen in *S. japonicum* and *S. mansoni,* respectively. They found that the circulating antigen is present in Golgi vesicles and in a thick layer of finely granular, moderately electron-dense material covering the gut epithelium. This antigen is known to be a proteoglycan composed primarily of N-acetylgalactosamine and D-glucuronic acid residues with minor amounts of amino acids. Galactose, glucose, N-acetylglucosamine and trace amounts of fucose and mannose are also present (Nash, 1977). Intense binding with SBA-gold to N-acetylgalactosamine residues in *S. japonicum* was anticipated in the present study. However, only weak labelling occurred on the surface coat of cytoplasmic projections. Further study is needed to clarify this unexpected result.

Ohara *et al.* (1985) demonstrated gut-associated antigen in *P. ohirai* with an immunofluorescent staining method and judged that there were some differences in antigenicity between juveniles and adults. Studies of *P. westermani* by Sugiyama *et al.* (1988) demonstrated that the gut-associated antigen has different molecular weights in larvae and adults. In the present study, differences between juveniles and adults of *P. ohirai* in the intensity of WGA-gold and RCA-I-gold labelling of gut-associated sugar residues also support the idea that juvenile and adult trematodes may differ in antigenicity.

Fujino *et al.* (1983) demonstrated N-acetyl-β-D-glucosaminidase and β-galactosidase in the gut epithelium of juvenile and adult *P. ohirai* and *P. westermani* by histochemical techniques. Strong reactions for these enzymes occurred on the brush border of the gut. The similar distribution of WGA-gold and RCA-I-gold on luminal cytoplasmic projections of the gut epithelium in this study suggests that N-acetylglucosamine and D-galactose are components of these enzymes.

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