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HAS ASIALOGLYCOPROTEIN RECEPTOR (ASGP-R) A ROLE TO PLAY IN BINDING AND PROCESSING OF DIFFERENT PARASITES?

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Liver asialoglycoprotein receptor (ASGP-R), which specifically recognizes and binds galactose and N-acetyl galactosamine, has been implicated in binding and endocytosis of glycoproteins. Therefore, the possibility that it may have a role in contacting and processing pathogenic organisms was investigated. The interaction in vitro between ASGP-R and surface oligosaccharide structures of Echinococcus granulosus and Trichinella spiralis was studied by immunohistochemical methods. Specific binding of ASGP-R to ligands on the surface protoscolex of E. granulosus was demonstrated. To the contrary, ASGP-R bound ligand(s) of T. spiralis stihocytes, while a staining reaction was absent from the parasite cuticle. These results show that ASGP-R could take part in binding to and retention of E. granulosus in liver cells, thus contributing to its hepatotropic activity.

Key words: asialoglycoprotein receptor (ASGP-R), Echinococcus granulosus, oligosaccharide ligand, Trichinella spiralis

INTRODUCTION

The asialoglycoprotein receptor (ASGP-R) was one of the first discovered mammalian lectins and the first that was implicated in a specific physiological function (Ashwell and Harford, 1982). It belongs to the family of C-type lectins, and the type II subgroup of endocytic receptors. ASGP-R specifically recognizes and binds galactose and N-acetylgalactosamine-terminated oligosaccharides (Maury, 1983). ASGP-R isolated from rabbit liver is a oligomer made up of two subunits with approximate molecular weights of 40 and 48 kD.

Functionally, ASGP-R is regarded as a prototype of the receptors that mediate binding, endocytosis and degradation of glycoproteins (Spiess, 1990). This role of ASGP-R was supported by the finding that alterations of ASGP-R activity were detected in some liver diseases. The receptor is also a potential target for hepatocyte-specific immunopathogenic reactions and for liver-specific delivery of therapeutic and diagnostic agents (Poralla *et. al.*, 1991). It is now possible to indicate several other processes where activities of the ASGP-R may play an important role. Current data suggest that ASGP-R is involved in clearance of soluble

immune complexes from the circulation, in mitogenesis of desialylated lymphocytes, in cell adhesion and transformation (Tomana *et al.*, 1988; Stahn *et al.*, 1996; Wu *et al.*, 1991).

Within the past few years, lectins have become a well-established means for studying varied aspects of cancerogenesis and metastasis (Hebert and Monsigny, 1997). ASGP-R is a receptor that is retained in tumoricidal cells, helping to increase metastasis in the liver. Also, several C-type lectins are an integral part of the innate defense system, so there is a possibility that ASGP-R may be connected with similar functions. Thus, it could be expected that ASGP-R has a role in contacting and further processing of pathogens containing appropriate oligosaccharides on their surfaces.

The aim of this paper was to examine the potential role of ASGP-R in contacting and processing of pathogens expressing Gal/GalNAc residues on their surface. This study was based on immunohistological localization of the oligosaccharide ligands for ASGP-R on the surface of the parasites selected.

The choice of parasites was made on the basis of their interaction with liver as a target organ. Echinococcosis (hydatid cyst disease) is a zoonotic infection caused by the parasitic tapeworm *Echinococcus granulosus*. Human infection follows accidental ingestion of ova present in dog faeces. The ova penetrate the intestinal wall and pass through the portal vein to the liver, lung and other tissues (Gvozdenović, 1962). Hydatid cysts can develop anywhere in the body, but the data demonstrate that two thirds occur in the liver and one quarter in the lung. A hydatid cyst may become calcareous forming a solid corpuscle inside the affected organ (Kazura and Williams, 1976).

To the contrary, the nematode *Trichinella spiralis* parasites only in striated muscle, but using blood and lymph systems this parasite could pass through all organs and tissues, including liver tissue (Takahashi, 1997). The larvae remodel the muscle cell turning it into a nurse cell and they reprogramme host genomic expression. There they encyst. The cysts are the infective stages which can be transmitted to any new host that eats cyst-containing meat.

MATERIALS AND METHODS

Preparation of rabbit ASGP-R

ASGP-R was purified from Triton X-100 extracts of acetone liver powders by affinity chromatography using two successive columns of D-Gal Sepharose, according to the previously described method (Hudgin *et al.*, 1974).

Tissue collection and processing

E. granulosus protoscoleces were isolated from the cysts of pig liver. They were injected into mouse intestine and then embedded in paraffin. Sections (5μm) were cut, air dried and stored at 4°C until use.

Muscle larvae of *T. spiralis* were isolated from infected rat meat after proteolytic digestion. *T. spiralis* larvae were injected into mouse intestine and embedded in paraffin. The sections of *T. spiralis* were prepared in the same way as *E. granulosus* sections.

Section types

Interactions between ASGP-R and surface structures of *Echinococcus granulosus or Trichinella spiralis* were examined on tissue sections embedded in paraffin. Three types of sections were used:

a: Sections of *E. granulosus* protoscoleces in mouse intestine.

b: Incapsulated muscle larvae of *T. spiralis* (the antigen was paraffin embedded rat tongue), present in nurse cells after transformation of the muscle cells.

c: Isolated *T. spiralis* larvae from striated muscles injected into mouse intestine. Muscle larvae were isolated from infected rat meat after proteolytic digestion, in an acid environment. Then, muscle larvae were cultured at least 20 hours in Dulbecco's Modified Eagle's Medium (DMEM), under sterile conditions.

Interactions between ASGP-R and parasites

Tissue sections were dewaxed in xylene during 5 min., then sections were treated with 9 5% alcohol during 10 minutes. After that, sections were rehydrated with three changes of PBS.

Immunostaining was performed on 5mm thick sections following dewaxing and rehydrating. Sections of *E. granulosus* as well as *T. spiralis* in muscle and intestine, were incubated with freshly isolated rabbit ASGP-R overnight at 4 °C. Primary polyclonal guinea pig anti-rabbit antibodies (anti-ASGP-R) were applied overnight at 4 °C at a dilution of 1:20, followed by incubation with sheep anti-guinea pig IgG diluted 1:32 (labeled with FITC) for 30 min at room temperature. Between each step, sections were washed three times in PBS. Sections were examined using a fluorescence microscope. PBS was used instead of rabbit ASGP-R in the control reaction.

RESULTS

In order to improve the specific reactions between freshly isolated ASGP-R and polyclonal guinea pig anti-rabbit IgG, the immuno blot technique was applied. The obtained reaction was very weak due to the low concentration of isolated rabbit ASGP-R (data not shown). However, these results allowed us to confirm that it is possible to use the polyclonal antibodies (obtained as a gift) in further examinations.

To that end purified ASGP-R and guinea pig anti-rabbit ASGP-R (to rabbit ASGP-R 40 kDa subunit) were used. On the *E. granulosus* sections clear fluorescence was observed at the surface of protoscolex tegumentum. Fluorescence was linear and surrounding the whole complex of protoscolex and this type of reaction was not observed in the control experiment (Figure 1).

Histological examination of incapsulated *T. spiralis* muscle larvae showed that ASGP-R, bound diffusely to the components of parasite organelles, probably to antigens of stihocytes, as well as digestive and reproductive systems (Figure 2).

Uniform binding was present at the inner as well as the outer aspect of the *T. spiralis* capsule. The staining reaction was absent from the surface of the parasite cuticle. Interaction between ASGP-R and T. spiralis muscle larvae was observed as a granular type of fluorescence on the surface of the cuticle (data not shown). Therefore, this type of fluorescence was also obtained in the control reaction.

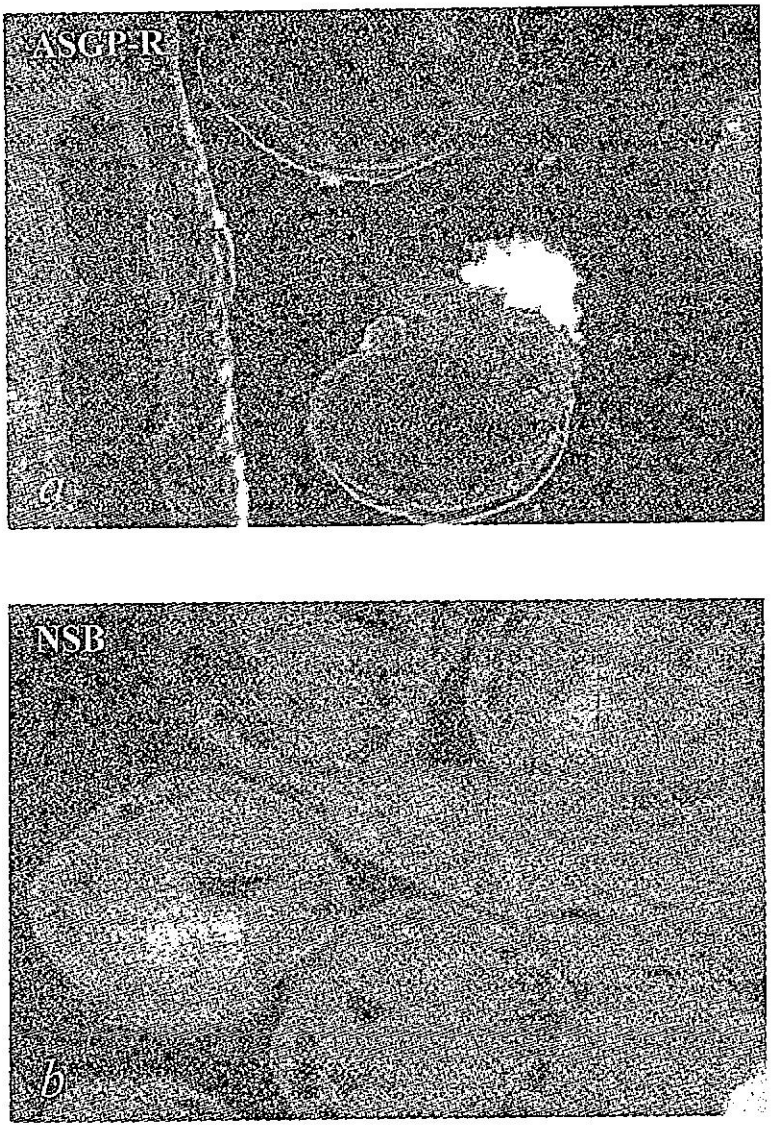

Figure 1. Binding of ASGP-R to surface protoscoleces of *Echinococcus granulosus*. (a) Paraffin sections of E. granulosus protoscoleces (from cysts formed in pig liver packed in mouse intestine) were incubated with ASGP-R (freshly isolated in our laboratory) overnight at 4°C. Primary antibodies were applied overnight at 4°C (dilution 1:20), followed by incubation with FITC labeled secondary antibodies (30 min. at room temperature). Sections were washed three times in PBS between the steps, mounted and examined under a fluorescence microscope. For negative control (b) the first antibody step was replaced with PBS.

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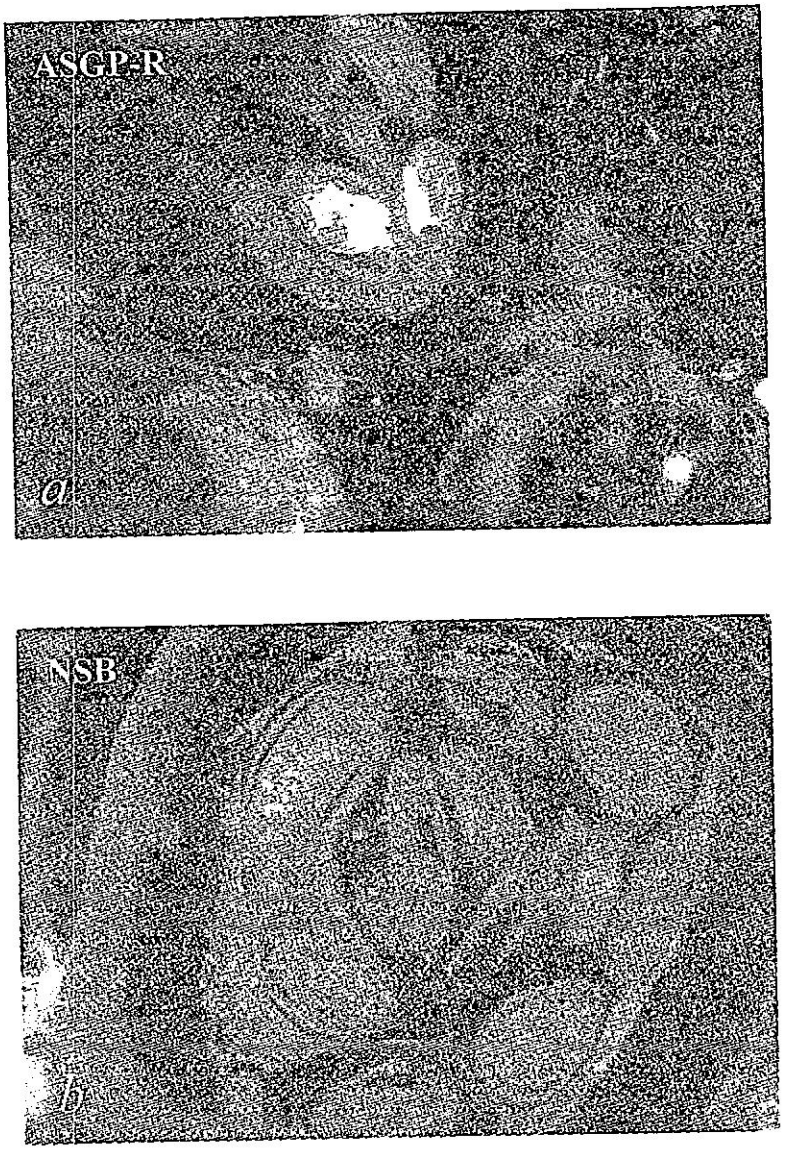

Figure 2. Binding of ASGP-R to incapsulated muscle larvae of *Trichinella spiralis* (paraffin sections of rat tongue muscle cells transformed into nurse cells). (a) The same experimental protocol was used as described for Fig.1, with an overnight incubation step with ASGP-R, followed by primary and secondary antisera at the same dilutions. For negative control (b) the first antibody step was replaced with PBS.

DISCUSSION

The presented histological results show that there is a difference between the oligosaccharide structures of *E. granulosus* and *T. spiralis* cell surfaces, as recognized by ASGP-R. *E. granulosus* ligands recognized by ASGP-R are characteristically arranged at the surface of protoscoleces tegumentum (Fig. 1a). This suggests that ASGP-R could possibly take part in the binding of this parasite to liver cells and consequent retention in the organ. A recent study (Khoo *et al.*, 1997) has shown that the *E. granulosus* cyst membrane contains complex-type N-glycans, N-acetylglucosamine, β-galactose and N-acetyllactosamine, which include the confirmed ligands of ASGP-R. Our results also confirmed that *E. granulosus* surface is covered with these oligosaccharide structures. In general, the ability of parasites to manipulate the host lectin system is well documented (Despommier, 1998), but in the case of *E. granulosus* it deserves further examination and experimental proof.

The case of *T. spiralis* is more complex. Interaction between ASGP-R and surface structures of incapsulated *T. spiralis* muscle larvae was observed. However, the staining reaction was absent from the surface of the parasite cuticle. Treating the parasite with proteolytic enzymes and acids, which simulates the environment of the host digestive system, led to the appearance of clear fluorescence on the surface of the cuticle. This type of fluorescence was also observed after the incubation of *T. spiralis* larvae only with primary anti-ASGP-R antibodies, so in this case we could not claim that there is a specific interaction between ASGP-R and oligosaccharide structures on the surface of isolated muscle larvae. It is known that the glycoconjugate composition of the parasite cuticle changes during infection (Dell *et al.*, 1999), which may represent parasite adaptation against the host immune system. In the normal life cycle of *T. spiralis* only the newborn larvae could be in contact with hepatic receptors, which stage was not studied here. The oligosaccharide structures of newborn larvae surface antigens is still unknown. There is some evidence that larvae are covered with glycoconjugates rich in mannose residues at this stage of infection (Morelle, 2000). An abundance of mannose residues leads to synthesis of mannose binding lectin (specific for mannose). Probably, these oligosaccharide structures do not allow interaction with hepatic ASGP-R, which is not specific for mannose residues.

Therefore, we suggest that the differential expression of specific sugars recognized by ASGP-R at the surface of the infectious agents studied may partly explan the hepatotropic activity of *E. granulosus*, which deserves further investigation.

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REFERENCES:

1. *Ashwell G, Harford J,* 1982, Carbohydrate-specific receptors of the liver. *Ann Rev Biochem,* 51, 531-54.
2. *Dell A, Haslam SM, Morris HR, Khoo KH,* 1999, Immunogenic glycoconjugates implicated in parasitic nematode diseases. *Biochim Biophys Acta,* 1455, 353-62.
3. *Despommier DD,* 1998, How does Trichinella spiralis make itself at home? *Parasitol Tod,* 14, 318-23.
4. *Gvozdenović M,* 1962, Helminti, In: Karakaševi*ƒ* B, editor, *Mikrobiologija i parazitologija,* Medicinska knjiga: Beograd-Zagreb, 699-726.
5. *Hebert E, Monsigny M,* 1997, Lack of modulation of the galectin-3 and asialoglycoprotein receptor transcription in hepatocarcinoma of transgenic mice. Biol of the Cell, 89, 467-73.
6. *Hudgin RL, Pricer WE, Ashwell G,* 1974, The isolation and properties of a rabbit liver binding protein specific for asialoglycoproteins. *J Biol Chem,* 249, 5536-43.
7. *Kazura JW, Williams JF,* 1976, An introduction to medical helmintology. In: Cohen S, Warren KS, editors. Immunology of the Parasitic Infections. Blackwell Scientific Publications.
8. *Khoo KH, Nieto A, Morris HR, Dell A,* 1997, Structural characterization of the N-glycans from Echinococcus granulosus hydatid cyst membrane and protoscoleces. *Mol Biochem Parasitol,* 86, 237-48.
9. *Maury CPJ,* 1983, Hepatic lectins: concept, biochemistry, and role. *Scand J Gastroenterol,* 18, 321-25.
10. *Morelle W, Haslam SM, Olivier V, Appleton JA, Morris HR, Dell A,* 2000, Phosphorylcholine-containing N-glycans of Trichinella spiralis: identification of multiantennary lacdiNAc structures. *Glycobiol,* 10, 941-50.
11. *Poralla T, Treichel U, Lohr H, Fleischer B,* 1991, The asialoglycoprotein receptor as target structure in autoimmune liver diseases. *Sem Liver Dis,* 11, 215-22.
12. *Spiess M,* 1990, The asialoglycoprotein receptor: A model for endocytic transport receptors. *Biochemistry,* 29, 10009-18.
13. *Stahn R, Schreiber J, Schafer H,* 1996, Specific inhibition of cell adhesion by exogenous glycoligands, In: Driessche EV, Rouge P, Beeckmans S, Bog-Hansen TC, editors, *Lectins: Biology, Biochemistry, Clinical Biochemistry,* Textop: Hellerup (Denmark), 11, 193-97.
14. *Takahashi Y,* 1997, Antigens of *Trichinella spiralis. Parasitol Tod,* 13, 104-06.
15. *Tomana M, Kulhavy R, Mestacky J,* 1988, Receptor-mediated binding and uptake of immunoglobulin A by human liver. *Gastroenterol,* 94, 762-70.
16. *Wu GY, Wu CH,* 1991, Targeted diagnosis and therapy using specific receptors and ligands. In: Wu GY, Wu CH, editors, *Liver Diseases,* New York: Marcel Dekker, 127-49.

DA LI ASIJALOGLIKOPROTEINSKI RECEPTOR (ASGP-R) IMA ULOGU U VEZIVANJU I PROCESOVANJU RAZLIČITIH PARAZITA ?

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SADRŽAJ

Asijaloglikoproteinski receptor (ASGP-R) jetre, koji specifično prepoznaje i vezuje galaktozu i N-acetilgalaktozamin, učestvuje u vezivanju i endocitozi glikoproteina. Zbog toga je u ovom radu ispitivana njegova potencijalna uloga u kontaktu i daljem procesovanju patogenih organizama. Interakcija između ASGP-R i površinskih oligosaharidnih struktura *Echinococcus granulosus* i *Trichinella spiralis* ispitivana je in vitro imunohistohemijskim metodama. Pokazano je specifično vezivanje ASGP-R za ligande na površini protoskoleksa *E. granulosus.* S druge strane, ASGP-R vezuje ligande stihocita *T. spiralis,* dok se bojena reakcija nije pojavila na površini kutikule parazita. Ovi rezultati pokazuju da bi ASGP-R mogao imati ulogu u vezivanju i zadržavanju *E. granulosus* u ćelijama jetre, što može doprineti njegovoj hepatotropnoj aktivnosti.