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LacdiNAc-Glycans Constitute a Parasite Pattern for Galectin-3-Mediated Immune Recognition[1]

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Although Galβ1–4GlcNAc (LacNAc) moieties are the most common constituents of N-linked glycans on vertebrate proteins, GalNAcβ1–4GlcNAc (LacdiNAc, LDN)-containing glycans are widespread in invertebrates, such as helminths. We postulated that LDN might be a molecular pattern for recognition of helminth parasites by the immune system. Using LDN-based affinity chromatography and mass spectrometry, we have identified galectin-3 as the major LDN-binding protein in macrophages. By contrast, LDN binding was not observed with galectin-1. Surface plasmon resonance (SPR) analysis and a solid phase binding assay demonstrated that galectin-3 binds directly to neoglycoconjugates carrying LDN glycans. In addition, galectin-3 bound to *Schistosoma mansoni* soluble egg Ags and a mAb against the LDN glycan inhibited this binding, suggesting that LDN glycans within *S. mansoni* soluble egg Ags contribute to galectin-3 binding. Immunocytochemistry demonstrated high levels of galectin-3 in liver granulomas of *S. mansoni*-infected hamsters, and a colocalization of galectin-3 and LDN glycans was observed on the parasite eggshells. Finally, we demonstrate that galectin-3 can mediate recognition and phagocytosis of LDN-coated particles by macrophages. These findings provide evidence that LDN-glycans constitute a parasite pattern for galectin-3-mediated immune recognition. *The Journal of Immunology*, 2004, 173: 1902–1907.

The innate immune system is equipped with a variety of receptors and soluble molecules that recognize invading pathogens by pathogen-associated molecular patterns (PAMPs).[3] PAMPs include a variety of structures, such as nucleic acids, lipids, proteins, and glycans, that are more or less selectively expressed by pathogens or pathogen-infected cells. Molecules involved in PAMP recognition include TLRs, scavenger receptors, and lectins (1–3). In general, these pattern recognition molecules have a relatively broad ligand selectivity and mediate immune functions including phagocytosis and Ag uptake for presentation.

Because many pathogens exhibit foreign patterns of glycosylation, as compared with their vertebrate host, the resultant glycans constitute attractive candidates for immune recognition. Indeed, a number of lectins have been identified, including mannose-binding lectin, the macrophage mannose receptor (3), and the dendritic cell-associated C-type lectin dendritic cell-specific ICAM-3-grabbing nonintegrin (4–6), that recognize mannose- and/or fucose-containing ligands from viruses, bacteria, parasites, and/or fungi. Recently, it was demonstrated that dendritic cells recognize Galβ1–4(Fucα1–3)GlcNAc (Lewis-x (Le^x), CD15) Ags on *Schistosoma mansoni* egg glycoproteins through interaction with dendritic cell-specific ICAM-3-grabbing nonintegrin (6). However, among helminth parasites, Le^x Ags are restricted to certain species (7), whereas many helminths, including schistosomes, express GalNAcβ1–4GlcNAc (LacdiNAc (LDN)) glycans, or their fucosylated derivatives containing GalNAcβ1–4(Fucα1–3)GlcNAc (LDNF) (8–11). LDN structures are abundant on the surfaces of worms and eggs, although the latter predominantly induce immune-mediated pathology (e.g., granuloma formation) during schistosomiasis (9). In addition, egg-derived Ags are potent immunogens and Abs against the LDN Ag have been detected in sera from infected human patients, mice, and primates (10, 12, 13).

Galectins (previously termed S-type lectins) constitute a family of mammalian β-galactoside binding lectins (14, 15). Galectin-3 (MAC-2) is composed of a C-terminal carbohydrate recognition domain (CRD), that is responsible for β-galactose recognition (16, 17), and an N-terminal domain consisting of multiple PGAYPG repeats, that mediates multimer formation (18). The structural basis for β-galactose recognition by the galectin-3 CRD has been established by x-ray crystallography (17). Galectin-3 is synthesized and secreted by myeloid cells, including macrophages, as well as several other cell types (19, 20), and binds to a variety of endogenous glycoprotein ligands, including cell surface receptors and extracellular matrix proteins (21, 22). It has also been identified as an IgE-binding protein (23) and Abs to galectin-3 have been shown to inhibit IgE-mediated cytotoxicity of eosinophils to adult schistosomes (24). Thus far, there is no evidence for a direct role of galectin-3 in schistosome recognition.

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[3] Abbreviations used in this paper: PAMP, pathogen-associated molecular pattern; CRD, C-terminal carbohydrate recognition domain; dig, digoxigenin; DSA, Datura stramonium agglutinin; GAM, goat anti-mouse; Le^x, Lewis-x; LN, LacNAc; LDN, LacdiNAc; LDNF, GalNAcβ1–4(Fucα1–3)GlcNAc; LDN-DF, GalNAcβ1–4 (Fucα1–2 Fucα1–3) GlcNAc; PAA, polyacrylamide; PO, peroxidase; RT, room temperature; SEA, *Schistosoma mansoni* soluble egg Ag; SPR, surface plasmon resonance.

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We postulated that LDN may constitute a parasite pattern for recognition by the immune system and attempted to identify putative LDN-binding lectins in macrophages. The results demonstrate that galectin-3 constitutes a major macrophage LDN-binding protein that is highly expressed in granulomas and colocalizes with LDN on eggshells during *S. mansoni* infection, and that galectin-3 can mediate LDN recognition and phagocytosis by macrophages. This implicates LDN as a parasite pattern for galectin-3-mediated immune recognition.

Materials and Methods

Animals and cells

Infection of golden hamsters with *S. mansoni* cercariae was performed as described previously (9). Animals were sacrificed after 7 wk and liver tissue was snap frozen in liquid nitrogen and stored at −80°C until further use. Male Wistar rats (6–12 wk of age) were obtained from Harlan/CPB (Horst, The Netherlands). Animals were maintained under conventional laboratory conditions allowing free access to food and water. Thioglycolate-elicited peritoneal macrophages were obtained by i.p. injection of 10 ml thioglycolate (Difco, Detroit, MI). After 4 days, the rats were sacrificed by CO_2 inhalation and peritoneal cells were harvested by peritoneal lavage with Opti-MEM (Invitrogen Life Technologies, Carlsbad, CA) and subsequently stimulated overnight with 100 ng/ml LPS (*Escherichia coli* 055: B5; Difco) in RPMI 1640 medium (Invitrogen Life Technologies) containing 10% FCS (Invitrogen Life Technologies), 100 μg/ml streptomycin, and 100 U/ml penicillin. The THP-1 human monocytic cell line, and the NR8383 rat alveolar macrophage NR8383 cell line (25) were cultured in RPMI 1640 medium containing 10% FCS and antibiotics.

Abs, neoglycoconjugates, and lectins

The following Abs were used: the anti-galectin-3 mAb A1D6 (mouse IgG) (26), the anti-GalNAcβ1–4 (Fucα1–2 Fucα1–3) GlcNAc (LDN-DF) mAb 114-5B1-A (mouse IgG1) (9), the anti-LDN mAb SMLDN1.1 (mouse IgM) (10), the anti-LDN mAb 273-3F2 (mouse IgM) (9), and the anti-LDNF mAb SMLDNF1 (mouse IgM) (11). Enzymatically synthesized carboxymethyl octyl-Le^x and LDN were coupled to BSA at similar molar ratios of 11–14 mol:mol as described (9). LacNAc (LN)-$(CH_2)_3$-NH_2 was coupled to BSA at a molar ratio of 9 mol:mol via diethylsquarate according to the method described in Ref. 27. The polyvalent neoglycoconjugates LN-polyacrylamide (PAA) and LDN-PAA, were from Syntesome (Munich, Germany) (saccharide 20% mol). Digoxigenin-labeled Datura stramonium agglutinin (DSA) (DSA-dig) and peroxidase-labeled anti-dig Abs (anti-dig-PO) were from Boehringer Mannheim (Indianapolis, IN). PO-conjugated $F(ab')_2$-fragments of goat anti-mouse IgM/IgG (GAM-PO) were from The Jackson Laboratory (Bar Harbor, ME).

Recombinant galectin-3 production

Strain BL21 (DE3) of *E. coli* was transformed with the rCPB30 plasmid encoding full-length hamster galectin-3 (28), and grown on Luria-Bertani agar containing 50 μg/ml ampicillin and 50 μg/ml chloramphenicol. Colonies were grown in Luria-Bertani medium for 2 days at 22°C; 0.4 mM isopropyl β-D-thiogalactoside was added after 4 h of culture. Galectin-3 was isolated essentially according to Mehul et al. (26). Briefly, pelleted cells were resuspended in 20 ml/L culture of lysis buffer (1 M Tris-HCl pH 7.4, containing 1 mM EDTA, 10 mM 2-ME, and 1 mM PMSF). The samples were frozen and thawed twice and centrifuged. Proteins were precipitated using 65% ammonium sulfate for 16 h and centrifuged. The pellet was resuspended in 50 mM Tris-HCl pH 7.2, containing 150 mM NaCl, 1 mM EDTA, and 2 mM 2-ME, and dialyzed three times against buffer without 2-ME. Galectin-3 was purified on a 1-ml lactosyl-Sepharose 4B column as described (26), and eluted with 50 mM Tris-HCl pH 7.2, containing 150 mM lactose, 150 mM NaCl, 1 mM EDTA, and 2 mM 2-ME. Purity and identity were analyzed by 12.5% SDS-PAGE and silver staining, and Western blotting using anti-galectin-3 mAb A1D6 (28) and PO-conjugated rabbit anti-mouse (DakoCytomation, Carpinteria, CA), respectively. Galectin-3 was estimated to be at least 95% pure. Before use, lactose and 2-ME were removed using a Bio-Gel P4 column (Bio-Rad, Veenendaal, The Netherlands). Biotinylation of galectin-3 was conducted in the presence of lactose using NHS-LC-LC-biotin (Perbio Science, Etten-Leur, The Netherlands), according to manufacturer's instructions.

Affinity purification and mass spectrometry of LN- and LDN-binding lectins

LN-$(CH_2)_3$-NH_2 was treated with jackbean β-galactosidase to remove the terminal galactose residue, and LDN was enzymatically synthesized using UDP-GalNAc and partially purified β4-*N*-acetylgalactosaminyltransferase from the albumen gland of *Lymnea stagnalis* as previously described (9). The structure was verified by ^{1}H-nuclear magnetic resonance spectroscopy as described (29). LN-$(CH_2)_3$-NH_2 and LDN-$(CH_2)_3$-NH_2 (5 μmol each) were then covalently coupled to 1 ml HiTrap columns (Pharmacia, Peapack, NJ) according to the manufacturer's instruction. NR8383 cells (10^9 total) and THP-1 cells (0.5×10^8 total) were washed, pelleted, and lysed by adding 3–4 ml of 50 mM Tris-HCl pH 7.2 buffer containing 1% Triton X-100, 150 mM NaCl, 2 mM $CaCl_2$, and protease inhibitors, split, and passed over the LN and LDN columns. The columns were washed with 10 bed volumes of buffer and eluted with buffer containing 200 mM lactose or GalNAc, respectively. Samples were desalted, boiled in loading buffer and run on a reducing 15% SDS-PAGE. Proteins were visualized by silver or Coomassie brilliant blue staining. Indicated protein bands were excised and trypsinized, and extracted peptides were subjected to MALDI-TOF analysis as described (30).

SPR analysis

The Le^x-, LN-, and LDN-BSA conjugates were used to generate a Biacore sensor chip (Stevenage, U.K.) as described in detail (12). Briefly, the neoglycoproteins were immobilized at a flow rate of 5 μl/ml in 10 mM sodium-acetate pH 4.0 onto a carboxymethylated dextran CM5 sensor chip (Biacore) by covalent amine coupling until an increase in ~4000 response units was observed. All analyses were performed using a Biacore 3000 instrument at a flow rate of 5 μl/ml at 25°C using 50 mM Tris-HCl pH 7.2 buffer containing 150 mM NaCl, 1 mM EDTA, and 2 mM 2-ME. Galectin-3 was injected at concentrations from 0 to 10 μM, and where indicated, 150 mM lactose was added to the samples. Injection times of samples were 2 min, followed by buffer injection to allow dissociation. The sensor chips were regenerated using a 2-min pulse of 100 mM HCl.

Solid phase binding assay

The solid phase binding assay was performed by coating neoglycoconjugates (0.1 μg/ml) or *S. mansoni* soluble egg Ags (SEA) (5 μg/ml) in ELISA plates overnight at 4°C, followed by blocking with 1% nonfat dried milk (Nutricia, The Netherlands) in TBS (50 mM Tris-HCl pH 7.4 containing 200 mM NaCl) for 30′ at room temperature (RT). After washing with TBS/0.1% Tween 20, biotinylated recombinant galectin-3 (1 μg/ml in TBS/0.1% Tween 20) was added, and the adhesion was performed for 60 min at RT. Unbound galectin-3 was washed away and binding was determined by streptavidin-PO conjugate. LDN glycans were detected with anti-LDN (mAb SMLDN1.1) and GAM-PO. DSA-dig was detected with anti-dig-PO. Where indicated, anti-glycan mAbs were used as competitive inhibitors. After blocking, coated SEA were preincubated with anti-glycan mAbs (SMLDN1.1, SMLDNF1, at concentration 0.1 mg/ml) for 30′ at RT before adding galectin-3. Coating of the neoglycoconjugates and SEA was confirmed in all experiments with appropriate lectins and glycan-specific Abs.

Immunohistochemistry

Immunohistochemistry was performed as previously described (31). Briefly, acetone-fixed cryostat sections (5 μm) were incubated with 10 μg/ml anti-galectin-3 mAb A1D6 in PBS containing 0.1% BSA (PBS/BSA). After washing, sections were incubated with Alexa594-conjugated GAM Ig in PBS/BSA containing 1% hamster serum. Access binding sites were then blocked with 20% normal mouse serum, and the sections were incubated with 10 μg/ml anti-LDN mAb 273-3F2 (mouse IgM (9)), washed, and subsequently incubated with FITC-conjugated $F(ab')_2$ rabbit anti-mouse IgM in the presence of 1% hamster serum. The sections were then mounted in Fluorostab (Cappel) and evaluated on a Nikon Eclipse E800 fluorescence microscope (Melville, NY).

Phagocytosis assay

Hundred microliters of a 1% suspension of carboxylated green fluorescent beads (0.75 μM; Polysciences, Warrington, PA) were incubated with 25 μg LDN-BSA overnight at 4°C on a rollerbank. Coated beads (0.00125%) were preincubated with 50 μg/ml galectin-3 in Opti-MEM containing 0.1% BSA for 30′ on ice; where indicated, 150 mM lactose was added. The beads were then added to washed LPS-stimulated thioglycolate-elicited peritoneal macrophages and incubated for 1 h at 37°C. After washing in PBS, the macrophages were detached using 4% lidocaine in PBS/BSA and phagocytosis was measured by flow cytometry using a FACScan (BD Biosciences, San Jose, CA). The percent of phagocytosis was calculated from

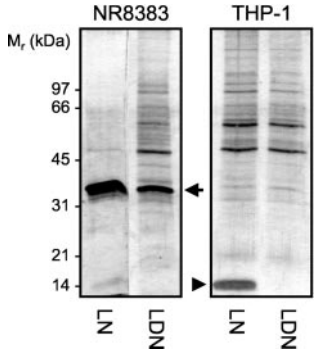

FIGURE 1. Identification of LDN-binding proteins from macrophages. Lysates from rat NR8383 macrophages and human THP-1 monocytic cells were subjected to affinity chromatography using columns containing immobilized LN and LDN. Binding proteins were eluted with lactose, electrophoresed by SDS-PAGE, and visualized by silver staining. The major (35-kDa) LDN-binding protein from NR8383 cells (arrow) and (14-kDa) LN-binding protein from THP-1 cells (arrowhead) were excised and identified by mass spectrometry as galectin-3 and galectin-1, respectively.

the mean fluorescence intensity of macrophages incubated with beads minus the mean fluorescence intensity of macrophages incubated without beads, and normalized to 100% using values obtained from macrophages incubated with BSA-coated beads.

Results

Galectin-3 is a major LDN-binding receptor in macrophages

To identify putative LDN-binding lectins, macrophage lysates from rat NR8383 and human THP-1 macrophage cell lines were passed over an affinity column containing immobilized LDN. Samples were passed over a control column with immobilized LN in parallel. Bound proteins were eluted with lactose, separated by SDS-PAGE, and visualized by silver staining (Fig. 1). Each cell type yielded a unique pattern of retained proteins. A major 35-kDa protein bound to both LN and LDN was present in NR8383 cell lysates, whereas a major 14-kDa protein bound to LN but not LDN in the THP-1 lysates. The major 35-kDa LDN-binding protein from NR8383 cells was excised from gel and trypsinized, and a selected peptide was subjected to mass spectrometric fragmentation. This yielded the peptide sequence GNDIAFHFNPR that corresponds to rat galectin-3 (23). The 14-kDa protein from THP-1 lysates bound by LN was identified by mass spectrometric fingerprinting as human galectin-1. Taken together, these results dem-

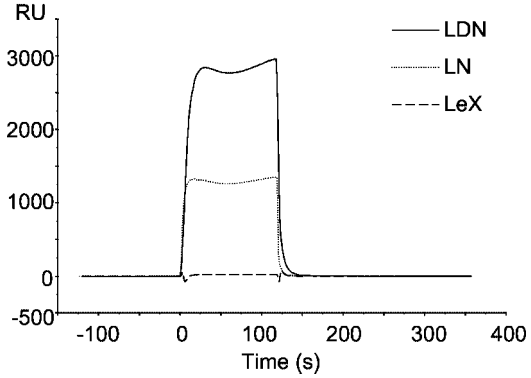

FIGURE 2. Direct binding of galectin-3 to LDN. SPR analysis of recombinant galectin-3 (10 μM) binding to sensor chips coated with BSA-LDN, BSA-LN, and BSA-Le^X. Note that galectin-3 binds to LDN and LN, but not to Le^X. Galectin-3 was added at time 0 and replaced by buffer at 120 s. RU, Response unit.

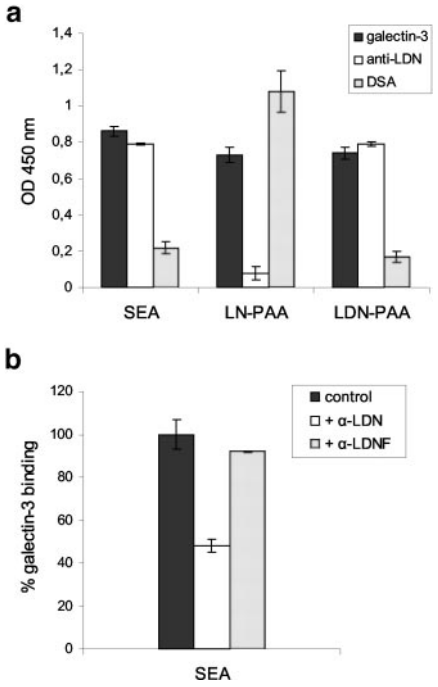

FIGURE 3. Galectin-3 binds to SEA and this involves LDN. *a*, Binding of galectin-3, anti-LDN mAb SMLDN1.1, and the LN-selective lectin DSA to immobilized SEA, LN-PAA, or LDN-PAA was evaluated by solid phase binding assay. Note that SEA, which contains LDN glycans but little or no LN glycans, binds galectin-3. *b*, Binding of galectin-3 to SEA is inhibited by anti-LDN mAb SMLDN1.1 but not by control anti-LDNF mAb. Data shown are from a typical experiment of three independent experiments conducted. Values represent means ± SD of duplicate measurements.

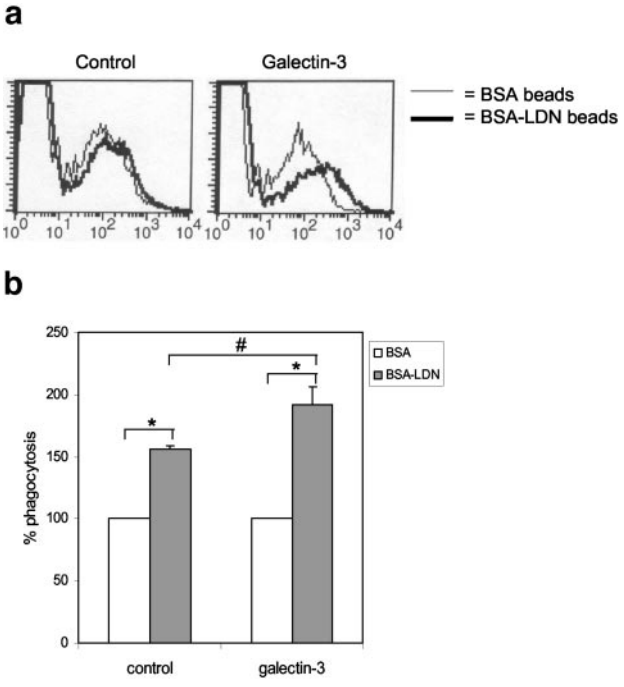

FIGURE 4. Galectin-3 mediates the binding and uptake of LDN-containing glycoconjugates by macrophages. Thioglycolate-elicited LPS-stimulated rat peritoneal macrophages were incubated with BSA- or BSA-LDN-coated fluorescent beads in the presence or absence of 50 μg/ml recombinant galectin-3. Cell-associated fluorescence was measured by flow cytometry. *a*, FACS plots of a representative experiment; *b*, mean ± SD of phagocytosis, expressed as a percentage from the phagocytosis of BSA beads, of three independent experiments. ∗, $p < 0.01$; #, $p < 0.05$ (Student's *t* test)

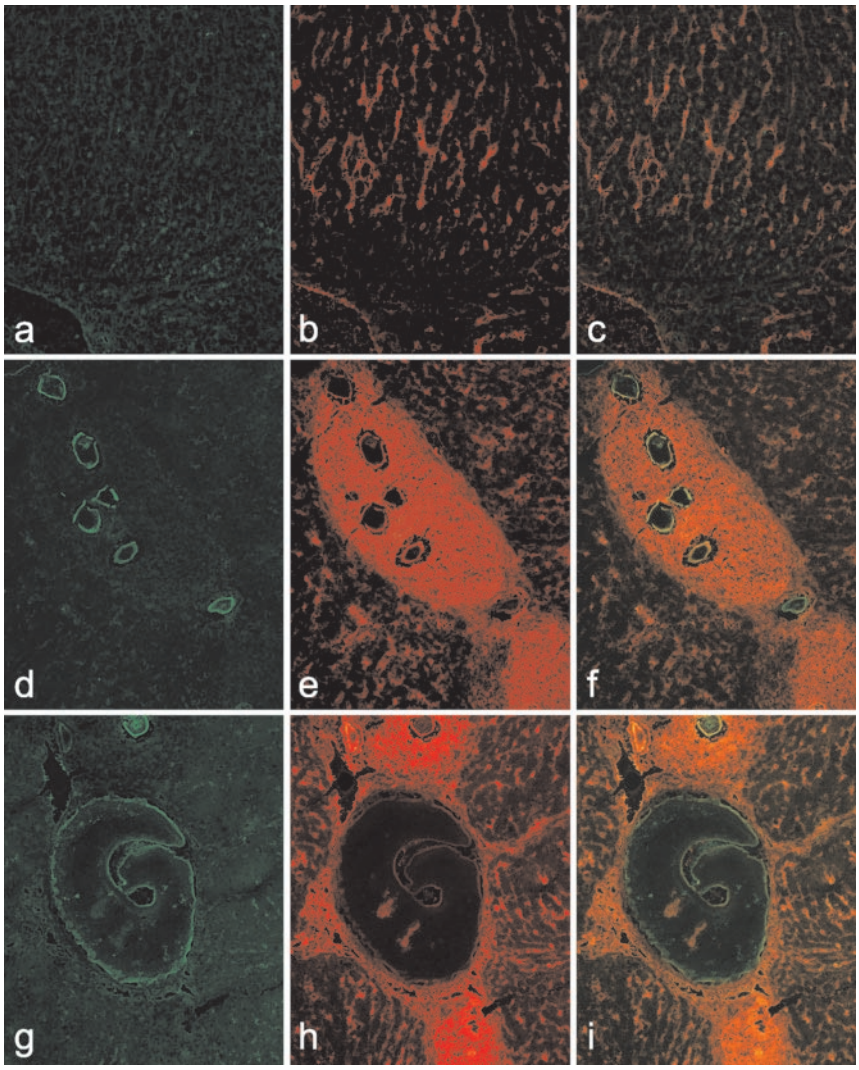

FIGURE 5. Localization of galectin-3 (red) and LDN (green) in the livers of normal (*a–c*) and *S. mansoni*-infected (*d–i*) hamsters. *a–c*, Liver from uninfected animal stained for galectin-3 and LDN. Note the galectin-3 staining in sinusoidal-lining cells and the absence of LDN staining. *d–f*, Liver granuloma with schistosome eggs. Note the intense galectin-3 staining in granulomas and the colocalization of galectin-3 with LDN on egg-shells. *g–i*, Liver granuloma with schistosome worm. Note the relatively weak galectin-3 staining at the worm surface.

onstrate that galectin-3 constitutes the major LDN-binding lectin in NR8383 macrophages, whereas galectin-1 does not bind LDN-glycans.

Galectin-3 directly binds LDN and SEA

To confirm that galectin-3 binds directly to LDN we analyzed the binding of purified recombinant hamster galectin-3 to LDN and to other related glycans by SPR using neoglycoconjugates. For proper comparison, the Biacore channels were coated with similar amounts of BSA-glycoconjugates in a molar glycan:BSA ratio of 10–12 mol:mol. This demonstrated that galectin-3 binds both to LN and LDN at all concentrations tested (1–10 μM, Fig. 2, and results not shown). However, a proper analysis of affinity was hampered because of the known oligomerization of galectin-3 (18). Binding of galectin-3 to BSA-LDN was completely prevented in the presence of 150 mM lactose, supporting involvement of the β-galactoside-binding CRD in LDN recognition. Consistent with previous findings (32), no binding to immobilized-BSA-Le^x was observed, thus demonstrating the strong negative influence of α1–3 fucosylation of the GlcNAc moiety on galectin-3 recognition. There was no detectable binding to the BSA-coated channel (data not shown).

In addition, the binding properties of biotinylated galectin-3 were analyzed in a solid phase binding assay. The results (Fig. 3*a*) show that galectin-3 recognizes neoglycoconjugates containing LN and LDN, respectively, as well as SEA, many of which express the LDN determinant (10). The binding of galectin-3 to SEA could be partially inhibited by the mAb SMLDN1.1 that recognizes LDN Ag (10), suggesting that galectin-3 indeed binds to LDN glycans within SEA. In contrast, binding was not inhibited by mAbs specific for the LDNF (11) (Fig. 3*b*), or the LDN-DF glycan Ag (9) (data not shown). To explore the possibility that galectin-3 also binds to possible LN moieties within SEA, we investigated whether the plant lectin DSA, that resembles galectin-3 in recognition of terminal LN moieties and internal ones in polylactosaminoglycan chains (33), but does not recognize LDN, could also bind SEA. No binding of DSA to SEA could be detected, indicating that unmodified terminal LN moieties and polylactosaminoglycan chains are not significant components of SEA.

Galectin-3 mediates uptake of LDN-containing glycoconjugates by macrophages

The SEA, which include LDN glycan Ags, constitute major antigenic determinants during *S. mansoni* infection (8, 34). Furthermore, granuloma formation around eggs depends on T cell-mediated immunity directed against such Ags (35). Importantly, recent findings in galectin-3-deficient mice indicate a supportive role for galectin-3 in granuloma formation (M. C. El-Cheikh, C. Takiya, and R. Chammas, personal communication). Therefore, it was

of interest to investigate whether galectin-3, which can form multimers via its N-terminal repeats (18), facilitates the binding and uptake of LDN-containing glycoconjugates by APCs, such as macrophages. Activated rat peritoneal macrophages were incubated with fluorescent latex beads coated with BSA-LDN, with and without addition of galectin-3. The uptake of BSA-LDN beads was significantly enhanced as compared with control BSA beads, in particular when exogenous galectin-3 was added (Fig. 4). By contrast, the phagocytosis of control BSA-coated beads that also was observed was not enhanced by addition of exogenous galectin-3. The uptake of BSA-LDN beads could be prevented to the level seen with BSA-coated beads by adding lactose (data not shown). It should be noted that the enhanced binding of BSA-LDN beads as compared with control beads, even in the absence of exogenous galectin-3, is in line with the expression of endogenous surface galectin-3 on activated macrophages (results not shown, see also Ref. 36). These results demonstrate that galectin-3 can act as an opsonin to facilitate the uptake of LDN-containing glycoconjugates by leukocytes and as such can mediate parasite-host cell interactions.

Galectin-3 is expressed in granulomas and colocalizes with LDN on eggshells during schistosomiasis

A possible interaction between galectin-3 and LDN glycans, the latter of which are expressed on the surface of worms and eggs shells during *S. mansoni* infection (9), was studied by double staining of livers of infected hamsters using mAbs against galectin-3 and LDN. In livers from healthy animals, a constitutive expression of galectin-3 was observed in sinusoidal lining cells (Fig. 5, *a–c*). In infected animals, high levels of galectin-3 were found in the granulomas surrounding eggs (Fig. 5, *d–f*) and worms (Fig. 5, *g–i*). In addition, galectin-3 colocalized with LDN expressed on the surface of eggs. Colocalization of LDN Ags and galectin-3 was also observed on the surfaces of worms, although the galectin-3 staining was less intense on the worm surface than on eggs.

Discussion

In the present study, we have investigated whether LDN-glycan Ags, which are commonly expressed by helminth parasites, constitute a pattern for immune recognition. We have identified galectin-3 as a major LDN-binding lectin in rat macrophages, and showed that it can mediate interactions between macrophages and schistosome LDN glycans. In addition, we found that galectin-3 is expressed in granulomas during schistosomiasis and colocalizes with LDN-glycans on eggshells. These results identify LDN as a parasite-associated molecular pattern for immune recognition.

Liver granuloma formation during *S. mansoni* infection is a major cause of pathology and is generally considered to be mainly the result of a T cell-mediated immunological reaction against parasite eggs (34, 37). The recent observation that granuloma formation during *S. mansoni* infection is significantly reduced in galectin-3-deficient mice (M. C. El-Cheikh, C. Takiya, R. Chammas, personal communication) provides direct evidence for a role of galectin-3 during parasite infection in vivo and suggests that galectin-3 plays a supportive role in granuloma formation. Clearly, more investigation is necessary to understand the exact role of galectin-3 in granuloma formation during schistosome infection. One possibility that is supported by our current evidence is that multimeric galectin-3 facilitates the uptake and presentation of LDN-containing Ags by macrophages (and/or other APCs). This uptake may trigger a more pronounced T cell response against the parasite, that may in turn promote granuloma formation. That a specific immune response against LDN-containing Ags does indeed occur during schistosomiasis is supported by the observation that Abs to LDN are present during experimental infection and in patients (10, 12, 13). Furthermore, soluble egg Ags have been detected in macrophages during *S. mansoni* infection (38). It will be interesting to evaluate cellular and humoral immune responses against soluble egg Ags during schistosomiasis in galectin-3-deficient mice.

It should be noted that recent evidence suggests that other schistosome-derived glycans, such as the difucosylated LDN-related oligosaccharide LDN-DF (39) and Le^x-containing glycoconjugates (5, 40), may also act as PAMPs to modulate macrophage cytokine production and dendritic cell maturation. However, the strict requirement for fucose in these studies points to recognition by different (i.e., galectin-unrelated) lectin-like receptors. Taken together, it seems likely that during helminth parasite infection different glycans, including LDN, contribute to pathogen recognition and the development of host immunity.

Although most galectins recognize LN moieties (32), the recognition of LDN glycans by a member of the galectin family has not been reported to our knowledge. The observation that lactose competes for LDN binding to galectin-3 indicates that LDN recognition involves the galectin-3 CRD. The inability of galectin-1 to recognize LDN as apparent from the affinity chromatography (Fig. 1) as well as from preliminary SPR experiments (C.H.H. and R.D.C, unpublished observations) demonstrates that LDN recognition is not a general property of galectin family members. The structural basis for binding of galactose by the galectin-1 and galectin-3 CRDs have been resolved by x-ray crystallography (41, 17), and this may provide a rational explanation for the observed difference in specificity between galectin-1 and -3. Indeed, our preliminary evaluation of these structures suggests that galectin-3 can accommodate the O-2 *N*-acetyl moiety of the GalNAc residue, while a bulky histidine (at position 52) in galectin-1 may prevent GalNac binding. Obviously, the exact structural explanation needs further investigation, but it seems clear, at least, that LDN binding is not common to all galectin family members. One interesting possibility is that galectin-3, by adopting LDN-binding activity during evolution, provided the innate immune system with a sensor for parasitic helminth infection, and likewise its host, with a selective evolutionary advantage.

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