Expression of Le\(^x\) antigen in *Schistosoma japonicum* and *S.haematobium* and immune responses to Le\(^x\) in infected animals: lack of Le\(^x\) expression in other trematodes and nematodes

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Adults of the human parasitic trematode *Schistosoma mansoni*, which causes hepatosplenic/intestinal complications in humans, synthesize glycoconjugates containing the Lewis x (Le\(^x\); Galβ1→4(Fucα1→3)GlcNAcβ1→R), but not sialyl Lewis x (sLe\(^x\); NeuAcα2→3Galβ1→4(Fucα1→3)GlcNAcβ1→R), antigen. We now report on our analyses of Le\(^x\) and sLe\(^x\) expression in *S.haematobium* and *S.japonicum*, which are two other major species of human schistosomes that cause disease, and the possible autoimmune to these antigens in infected individuals. Antigen expression was evaluated by both ELISA and Western blot analyses of detergent extracts of parasites using monoclonal antibodies. Several high molecular weight glycoproteins in both *S.haematobium* and *S.japonicum* contain the Le\(^x\) antigen, but no sialyl Le\(^x\) antigen was detected. In addition, sera from humans and rodents infected with *S.haematobium* and *S.japonicum* contain antibodies reactive with Le\(^x\). These results led us to investigate whether Le\(^x\) antigens are expressed in other helminths, including the parasitic trematode *Fasciola hepatica*, the parasitic nematode *Dirofilaria immitis* (dog heartworm), the rumen nematode *Haemonchus contortus*, and the free-living nematode *Caenorhabditis elegans*. Neither Le\(^x\) nor sialyl-Le\(^x\) is detectable in these other helminths. Furthermore, none of the helminths, including schistosomes, express Le\(^x\), Le\(^b\), Le\(^y\), or the H-type 1 antigen. However, several glycoproteins from all helminths analyzed are bound by *Lotus tetragonolobus* agglutinin, which binds Fucα1→3GlcNAc, and *Wisteria floribunda* agglutinin, which binds GalNAcβ1→4GlcNAc (lactoNAc or LDN). Thus, schistosomes may be unique among helminths in expressing the Le\(^x\) antigen, whereas many different helminths may express α1,3-fucosylated glycans and the LDN motif.

**Key words:** Lewis x antigen/*Schistosoma mansoni*/*Schistosoma haematobium*/*Schistosoma japonicum*/Haemonchus contortus*/Dirofilaria immitis*/Fasciola hepatica*/Caenorhabditis elegans

**Introduction**

Adult *S.mansoni* express the Lewis x (Le\(^x\); Galβ1→4(Fucα1→3)GlcNAcβ1→R), but not the sialyl Lewis x (sLe\(^x\); NeuAcα2→3Galβ1→4(Fucα1→3)GlcNAcβ1→R), antigen on both membrane and secreted glycoproteins (Ko et al., 1990; Srivatsan et al., 1992a; Van Dam et al., 1994; Cummings and Nyame, 1996). Glycans containing the Le\(^x\) antigen constitute a major target for host immune response during infection. Cytolytic IgM and IgG antibodies reactive to Le\(^x\) are generated in both infected humans and animals (Ko et al., 1990; Nyame et al., 1996, 1997; Van Dam et al., 1997). Expression of Le\(^x\) is developmentally regulated in *S.mansoni*, where the antigen is detectable on *S.mansoni* schistosomula and adults, but it appears to be absent from cercariae (Koster and Strand, 1994).

Adult *S.mansoni* also synthesize N-glycans containing the lactoNac sequence (LDN; GalNAcβ1→4GlcNAc-R) and LDN sequences in which GlcNAc residues are α1,3-fucosylated to generate LDNF (GalNAcβ1→4(Fucα1→3)GlcNAc-R) (Srivatsan et al., 1992b). LDNF-related structures have also been described in cercarial and egg glycoconjugates of *S.mansoni* (Kho et al., 1995, 1997a,b). Interestingly, LDNF-related structures and Fucα1→3GlcNAc linkages, but not the Le\(^x\) antigen, have been identified in microfilaria of the parasitic nematode *D.immitis* (dog heartworm) (Kang et al., 1993) and in adults of the ruminant nematode *H.contortus* (Haslam et al., 1996; R.A.De-Bose-Boyd, A.K.Nyame, D.P.Jasmer, and R.D.Cummings, unpublished observations). Thus, while LDN and LDNF may be common forms of N-glycosylation for both trematodes (flatworms) and nematodes (roundworms), Le\(^x\) expression may be restricted to members of the trematode family, and possibly only to schistosomes.

Fucosylated glycans have been shown in mammalian systems to be important recognition determinants in cell–cell interactions. In human neutrophils, α1,3-fucosylated glycans in cell surface glycoproteins are required for selectin-mediated adhesion to the endothelium (McEver and Cummings, 1997). The roles of Le\(^x\), LDN, and LDNF glycans in *S.mansoni* are not known. Furthermore, the distribution of Le\(^x\), sLe\(^x\), LDN, LDNF, and other biologically important fucosylated glycans among schistosomes and other helminths has not been described.

We have investigated the expression of Le\(^x\), sLe\(^x\), other Lewis antigens, LDN, and LDNF in adults of two other major human schistosome species, *S.haematobium* and *S.japonicum*, and in a group of selected flatworms and roundworms including *D.immitis*, *H.contortus*, the related parasitic flatworm *F.hepatica*, and the free-living roundworm *C.elegans*. Our results demonstrate that Le\(^x\) expression is common among schistosomes, but absent in other helminths. The presence of Le\(^x\) antigen in schistosomes is associated with anti-Le\(^x\) responses in infected humans and animals. Conversely, our new results, combined with recent studies on the glycosyltransferase activities in various helminths
(R.A.DeBose-Boyd, A.K.Nyame, D.P.Jasmer, and R.D.Cummings, unpublished observations), indicate that all helminths can generate LDN and LDN-related structures. These findings suggest that α1,3-fucosylation of glycans may be important in developmental processes for all the worms, whereas Le^x expression may be specifically involved in schistosomiasis.

**Results**

**Le^x antigen expression in S.haematobium and S.japonicum and lack of expression in other helminths**

To determine whether Le^x and/or sLe^x is expressed by other schistosome species and helminths in general, extracts of *S.haematobium* and *S.japonicum*, *F.hepatica*, *D.immitis*, *C.elegans*, and *C. elegans* were analyzed by ELISA using mAb CD-15 to Le^x and mAb CSLEX-1 to sLe^x. As positive controls, extracts of COS7 cells expressing human FTIII (COS7/FTIII), which express both Le^x and sLe^x determinants (Kukowska-Latallo et al., 1990), and extracts of *S.mansoni*, which express Le^x, but not sLe^x, were also analyzed. Extracts of wild-type COS7 cells, which lack Le^x and sLe^x determinants, were analyzed as negative controls (Nyame et al., 1996; R.A.DeBose-Boyd, A.K.Nyame, D.P.Jasmer, and R.D.Cummings, unpublished observations). Le^x was detected in extracts of both *S.haematobium* and *S.japonicum*, but not in extracts of *F.hepatica*, *C.cortortus*, *D.immitis*, or *C.elegans* (Figure 1A). sLe^x expression was not detectable in extracts of any of the helminths (Figure 1B), indicating the absence of sLe^x antigens from both flatworms and roundworms.

In a complementary approach, Western blot analyses for expression of Le^x were also performed using total glycoproteins from these organisms. Extracts of *S.mansoni*, COS7/FTIV cells and wild type COS7 cells were analyzed as controls. Le^x expression was observed in a number of high molecular weight glycoproteins in *S.haematobium* and *S.japonicum* (Figure 2A). (These gels were intentionally overstained to enhance the detectability of possible Le^x expression in the helminths other than schistosomes.) The mAb also stained many bands from *S.mansoni* and COS7/FTIV cells. In contrast, Le^x expression was not detected in glycoproteins from *C.elegans*, *H.cortortus*, *D.immitis*, or *C.elegans* (Figure 2B). These results confirm that both *S.haematobium* and *S.japonicum* express Le^x antigens and demonstrate that the determinants are distributed on a group of high molecular weight glycoproteins. Furthermore, the results indicate that Le^x is not common among flatworms or roundworms, but rather its expression appears to be limited to schistosomes.

**Absence of other Lewis antigens on schistosome species and other helminths**

We next examined whether human schistosomes and the other helminths synthesize glycans containing other Lewis blood group antigens. Extracts of helminths were analyzed by ELISA using mAbs to Le^a*, Le^b*, Le^y*, and blood group H determinants. Human milk derived bile salt-activated lipase (BAL), which contains many of the human blood group antigens (Wang et al., 1995; Landberg et al., 1997), and extracts of wild-type COS7 cells were analyzed as positive and negative controls, respectively. The extracts of the helminths and COS7 cells do not express Le^a*, Le^b*, or Le^y*, whereas all three mAbs reacted with BAL (Figure 3). In a similar approach we were unable to detect reactivity of the anti-H mAb toward any of the helminths or COS7 cells, whereas the mAb did react with BAL (data not shown). These results show that none of the helminths examined express Le^a*, Le^b*, Le^y*, or H-type-1 glycans.

**Presence of anti-Le^x Abs in rodents infected with S.haematobium and S.japonicum**

We previously observed that sera from animals infected with *S.mansoni* contained IgM and IgG responses to Le^x-containing glycans (Nyame et al., 1997). We tested whether a similar response exists in animals infected with *S.haematobium* and *S.japonicum*. For this analysis a neoglycoprotein prepared from BSA was used. BSA is the carrier in preparations of neoglycoproteins because it is devoid of carbohydrate (Stowell and Lee, 1980). LNFPIII-BSA, which contains the Le^x antigen, was used as the target. The neoglycoprotein was coated onto microtiter plates and incubated with serially diluted sera from infected or uninfected mice and hamsters. The results demonstrate that the sera from mice infected with *S.japonicum* and hamsters infected with *S.haematobium*, but not sera from uninfected animals, contain IgM and IgG reactive with LNFPIII-BSA (Figure 4). These results demonstrate that infectivity with *S.haematobium* and *S.japonicum* induce immune responses in animals to Le^x.
antigens as observed for \textit{S.mansoni} infections. The specificity of the immune response to Lewis antigens was assessed in ELISA using the neoglycoproteins sLe\textsubscript{x}-BSA, LNnT-BSA, and LNFPII-BSA. Neither IgM nor IgG reactivity was observed in the sera from infected animals toward these other targets, demonstrating that the autoimmunity in infected animals is specific toward the Le\textsuperscript{x} antigen (Figure 5).

\section*{Presence of anti-Le\textsuperscript{x} Abs in humans infected with \textit{S.haematobium} and \textit{S.japonicum}}

We previously reported that sera from humans infected with \textit{S.mansoni} contained antibodies to the Le\textsuperscript{x} antigen (Nyame \textit{et al.}, 1996). To determine whether this autoimmune response also exists in humans infected with the other schistosome species, pooled sera from uninfected humans or humans infected with either \textit{S.haematobium} or \textit{S.japonicum} were incubated with either wild-type COS7 cells, which lack Le\textsuperscript{x} antigens, or COS7/FTIV cells, which express Le\textsuperscript{x}, but not sLe\textsuperscript{x}, antigens. The cells were analyzed for bound Abs by incubation with goat anti-human IgM-FITC conjugate followed by FACS analysis on a flow cytometer. Pooled sera from \textit{S.mansoni} infected humans was also analyzed as a positive control. Sera from humans infected with all the three schistosome species stained COS/FTIV cells, while staining of control COS7 cells was weak (Figure 6). COS/FTIV and COS7 cells were only weakly stained by sera from uninfected humans (Figure 5). Thus, like \textit{S.mansoni}, Le\textsuperscript{x} glycans of \textit{S.haematobium} and \textit{S.japonicum} are immunogenic in infected humans.

\section*{Evidence for expression of LDN and \(\alpha_1,3\)-fucosylated glycans in glycoproteins from all helminths analyzed}

Both \textit{S.mansoni} and \textit{D. immitis} synthesize glycoconjugates containing the LDN motif GalNAc\(\beta_1\rightarrow4\)GlcNAc and the GlcNAc residue in many of the LDN residues are \(\alpha_1,3\)-fucosylated to generate LDNF (Srivatsan \textit{et al.}, 1992; Kang \textit{et al.}, 1993). In recent studies we found that both \textit{H.contortus} and \textit{C.elegans} contain an \(\alpha_1,3\)-fucosyltransferase (\(\alpha_1,3\)FT) activity capable of transferring Fuc to GlcNAc residues in either lactosamine Gal\(\beta_1\rightarrow4\)GlcNAc or LDN acceptors (R.A.DeBose-Boyd, A.K.Nyame, D.P.Jasmer, and R.D.Cummings, unpublished observations). In addition, we detected the \(\beta_1,4\)-GalNac-galactosaminytransferase (\(\beta_1,4\)GalNAcT) capable of synthesizing LDN in extracts of \textit{S.mansoni} (Srivatsan \textit{et al.}, 1994), \textit{H.contortus} and \textit{C.elegans}. However, we did not detect \(\beta_1,4\)-galactosyltransferase (\(\beta_1,4\)GalT) in extracts of these latter two helminths (R.A.DeBose-Boyd, A.K.Nyame, D.P.Jasmer, and R.D.Cummings, unpublished observations). The results suggest that helminths may generally be able to synthesize LDN-type structures and LDNF, but only schistosomes may be unable to synthesize the Le\textsuperscript{x} antigen, because schistosomes contain a \(\beta_1,4\)GalT (Rivera-Marrero and Cummings, 1990).

To further investigate this possibility, we utilized two plant lectins that bind to glycans containing the LDN and LDNF motifs. \textit{Lotus tetragonolobus} agglutinin (LTA) binds to these glycans.
Absence of Leα, Leβ, and Leγ from trematodes and nematodes. Microtiter wells were coated with extracts from the indicated helminths and analyzed for Leα, Leβ, and Leγ antigens by ELISA using mAb specific for the three glycans. Human bile-activated lipase (BAL) was analyzed as a positive control, while extracts of COS7 cells were analyzed as negative controls. The ELISAs were performed in triplicate and the results represent averages of the three determinations.

Discussion

Our results demonstrate that adult *S. haematobium* and *S. japonicum* synthesize high molecular weight glycoproteins containing Leα glycans. Expression of the Leα antigen is highly specific, since none of the three human schistosomes express the sLeα, Leα, Leβ, Leγ, or blood group H-type 1 antigens. In addition, the results indicate that animals and humans infected with *S. haematobium* and *S. japonicum* exhibit specific immune responses to the Leα antigen, as is observed for infections with *S. mansoni* (Ko et al., 1990; Nyame et al., 1996, 1997; Van Dam et al., 1996).

Although parasitic helminths represent a sizable fraction of all animal parasites, very little is known about the overall structures of their glycoconjugates, despite extensive evidence that glycans from parasitic helminths are immunogenic. Most of the emphasis to date has been on schistosomes, where they have been found to contain polylactosamine sequences and Leα-antigens on complex-type N- and O-glycans (Srivatsan et al., 1992a; Van Dam et al., 1994), LDN- and LDNF-containing structures on complex-type N-glycans (Nyame et al., 1989; Srivatsan et al., 1992b), high mannose-type N-glycans (Nyame et al., 1988a) and glycans containing an unusual trisaccharide repeating structure that is fucosylated (Levery et al., 1992; Kho et al., 1995, 1997a).

Interestingly, some of the 1,3-fucosyl residues in *S. mansoni* egg glycolipids are 1,2-fucosylated to generate Fuc1→2Fuc1→3GlcNAc structures, but these difucosylated structures are lacking in *S. japonicum* (Kho et al., 1997a). In addition, unusual core structures containing xylose and α1-3- and α1-6-linked fucosyl residue were described in *S. mansoni* and *S. japonicum* and the relative amounts of these modifications differ between the two species (Kho et al., 1997b).

The synthesis of these structures in *S. mansoni* and other schistosomes is consistent with their known complement of glycosyltransferases. Schistosomes contain a β1,4GalNACT (Neeleman et al., 1994; Srivatsan et al., 1994), an α1,3FT (DeBose-Boyd et al., 1996), and a β1,4Galβ1,3GalNAcT (Levery et al., 1992; Hsu et al., 1997). Despite these differences, schistosomes share a common β1,4GalNACT that is responsible for the synthesis of the Leα antigen. Furthermore, the synthesis of Leα antigens in *S. mansoni* and *S. japonicum* is likely to be mediated by a single enzyme, which is consistent with the expression of a single β1,4GalNACT in these species.
Fig. 5. Specificity of anti-Le^a antibody responses in infected rodents. Pooled sera from S.japonicum infected mice and S.haematobium infected hamsters were serially diluted and analyzed for either IgM or IgG antibody reactivity toward LNFPIL-BSA (▲), LNFPIL-BSA (●), sialyl Le^a-BSA (□), and LNnT-BSA (▲) by ELISA, as described in Materials and methods. Each ELISA was done in triplicate, and the results represent averages of the three determinations.

et al., 1996) and a β1,4GalT (Rivera-Marrero and Cummings, 1990). The β1,4GalNAcT is required for LDN biosynthesis, whereas the β1,4GalT is required for N-acetyllactosamine biosynthesis, which is the precursor for synthesis of the Le^a antigen. However, the expression of a functional β1,4GalT may be the limiting factor that precludes expression of Le^a in most helminths. For example, extracts of adult H.contortus contain both α1,3FT and β1,4GalNAcT activity, but β1,4GalT activity is not detectable (R.A.DeBose-Boyd, A.K.Nyame, D.P.Jasmer, and R.D.Cummings, in preparation). The expression of the β1,4GalT activity in schistosomes may be developmentally regulated, since little galactose is incorporated into glycoconjugates by transformed schistosomula, whereas galactose incorporation is abundant in adult schistosomes (Nyame et al., 1988b). Thus, developmentally regulated expression of the β1,4GalT, rather than the α1,3FT, may be responsible for the observed developmentally regulated expression of the Le^a antigen in schistosomes (Koster and Strand, 1994). The observation that all the helminths tested in our study contain glycoproteins recognized by WFA and LTA suggests that all helminths synthesize LDN, rather than N-acetyllactosaminyl-based structures and these contain α1,3-fucosylated GlcNAc residues. However, detailed carbohydrate structural analyses are required for glycoconjugates from all these helminths before this can be definitively established.

Very little is known about glycoconjugate structures in helminths other than schistosomes. The only detailed structural analysis reported for C.elegans glycoconjugates was concerned with neutral glycosphingolipids, where they were shown to contain unusual core structures with Glc, Man and GlcNAc (Gerdt et al., 1997). It has been reported that N-glycans in H. contortus contain unusual fucosylated core structures with both Fucα1→3GlcNAc and Fucα1→6GlcNAc linkages (Haslam et al., 1996). These unusual structures may be related to the observation that H. contortus produce several gut-derived galactose-containing antigens (H-gal-GP; Redmond et al., 1997) and membrane glycoproteins that contain highly antigenic glycans (Jasmer and McGuire, 1991; Jasmer et al., 1993; Andrews et al., 1995). D.immitis microfilaria are known to synthesize complex-type N-glycans containing the LDNF motif (Kang et al., 1993), but nothing is known about the glycan structures in adult organisms. Finally, there are no reports on the structural definition of glycoconjugates in F.hepatica.

In previous studies, it was observed that S.mansoni glycans lack sialic acid residues (Nyame et al., 1987; Robertson and Cain, 1997).
Fig. 6. Presence of anti-Le\(^x\) antibodies in sera of S.japonicum and S.haematobium infected humans. Pooled sera from S.japonicum and S.haematobium infected humans were diluted 1:20 and incubated with COSFTIV or COS7 cells as described in Materials and methods. Bound antibodies were detected by flow cytometry following an incubation with goat anti-mouse IgM-FITC. Pooled sera from uninfected humans and S.mansoni infected humans were analyzed as negative and positive controls, respectively.

1985). Consistent with this observation, neither S.mansoni nor the other schistosome species, express sLe\(^x\) determinants (this study and Srivatsan et al., 1992). The current findings suggests that sialic acid may be lacking in schistosome glycans in general.

The functions of Le\(^x\) glycans and other \(\alpha_1,3\)-fucosylated glycans in schistosomes and the other helminths are not known. In mammalian systems, fucosylated glycans, and in particular sLe\(^x\), are involved in leukocyte-selectin interactions in the inflammatory response (McEver and Cummings, 1997; Varki, 1997). sLe\(^x\) is a critical component of the glycoprotein PSGL-1 on neutrophils recognized by P- and E-selectins. Le\(^x\) and its derivatives occur on many human tissues and cells, in addition to leukocytes, but their functions in these other tissues are not known (Macher and Beckstead, 1990; Ohta et al., 1993; Satoh and Kim, 1994; McEver and Cummings, 1997).

The possibility that \(\alpha_1,3\)-fucosylated glycans in helminths may interact directly with selectins has been explored recently. Schistosome eggs, which express a variety of fucosylated glycans, interact with L-selectin, but whether binding involves Le\(^x\) or a related fucose-containing glycan, is yet not known (El Ridi et al., 1996). In addition, oligosaccharides containing the LDNF structure bind with low affinity to E-selectin (Grennel et al., 1994), but whether the LDNF structures in helminthic glycoconjugates bind to E-selectin has not been investigated. It is also possible that Le\(^x\) of schistosomes and the LDNF-containing glycans in all helminths interact with an unknown carbohydrate-binding protein(s) during parasite invasion or development in infected humans. In addition to possible roles in adhesion, fucose-containing glycans may alter the host immune response. There is evidence that Le\(^x\) glycans may alter cellular immunity in infected hosts, by facilitating a shift from Th1 to Th2 response (Velupillai and Harn, 1994; Palanivel et al., 1996). Whether these phenomena occur in infections with S.haematobium and S.japonicum is not yet known.

On the basis of the generation of cytolytic autoantibodies to Le\(^x\) glycans in infected humans and primates, it has been predicted that an autoimmune disorder may accompany severe and chronic schistosomiasis in humans infected with S.mansoni (Nyame et al., 1996; Van Dam et al., 1996). The presence of anti-Le\(^x\) antibodies in S.haematobium and S.japonicum infected humans raises the potential for an autoimmune disorder during infections by all human schistosome species. In addition to their cytolytic activity, antibodies to Le\(^x\) can have profound effects on human neutrophil function (Skubitz et al., 1985; Skubitz and Snook, 1987). Schistosome-derived Le\(^x\) antigens may also be involved in antibody-dependent cell-mediated cytotoxicity (Trottein et al., 1997). Further studies are needed to determine if the anti-Le\(^x\) response plays an important role in the pathology of schistosomiasis. Because of the specificity we have observed in the expression of Le\(^x\) antigen by schistosomes, the presence of anti-Le\(^x\) might be useful in diagnosing and distinguishing schistosome infections from other parasitic infections. This possibility should be investigated in the future by examining the
**Materials and methods**

**Parasites**

Adult *S. mansoni* and *S. haematobium* were derived from LVG hamsters infected for 8 weeks and 12 weeks, respectively, with cercariae of the two schistosome species. Adult *S. japonicum* were obtained from CD1 female mice infected for 8 wk with *S. japonicum* cercariae. Parasites were recovered from the rodents by portal perfusion. *D. immitis* adults were obtained from NIH Repository at the University of Georgia. Frozen *C. elegans* adults were the kind gift of Dr. James B. Rand of Oklahoma Medical Research Foundation. *H. contortus* adults were derived as described previously (Jasmer and McGuire, 1991). *F. hepatica* adults were derived from the livers of an infected cow obtained from a local abattoir.
Preparation of parasite extracts

Extracts of adult parasites were prepared in a solubilization buffer of phosphate-buffered saline (PBS, pH 7.4) containing protease inhibitors; EDTA (37 µg/ml), trypsin inhibitor (10 µg/ml), PMSF (1000 µM), apronitin (2 µg/ml), leupeptin (0.5 µg/ml), pepstatin (0.7 µg/ml), and 3,4-dichloroisocoumarin (200 µM). *S. mansoni, S. haematobium, S. japonicum, and E. hepatica* were disrupted by direct sonicatation on 4°C on a Branson Sonifier using three, 30 s bursts. *H. contortus, D. immitis,* and *C. elegans* were first disrupted on ice with Biohomogenizer (model M133/1281–0, Biospec Products, Inc., Bartlesville, OK) using three, 30 s pulses. The homogenates were disrupted further by sonication as described above. The homogenates were subsequently adjusted to 1 % Triton X-100 and left on ice for 30 min to allow complete solubilization of proteins. The homogenates were first centrifuged at 3000 r.p.m. for 30 min at 4°C, and supernatant fractions were centrifuged further at 50,000 r.p.m. for 1 h at 4°C. The supernatants were recovered and used directly or aliquots were prepared and stored at -80°C.

Preparation of cell extracts

COS7/FTIII and COS7/FTIV cells were generated by stably transfecting wild type COS7 cells with pcDNA I plasmids containing a cDNA insert for either human fucosyltransferase III or IV and a neomycin-selectable marker (Kukowska-Latallo et al., 1990; Kumar et al., 1991; Lowe et al., 1991). The COS7/FTIII and COS7/FTIV cells were maintained in Dulbecco’s Modified Eagle (DME) media containing 10% fetal bovine serum (FBS), 2 mM glutamine, 100 µg/ml penicillin, 100 µg/ml streptomycin, and 400 µg/ml neomycin (G418). Wild-type COS7 cells were maintained in the same media without G418. All cell cultures were maintained at 37°C and 5% CO2.

Cell culture

COS7, COS7/FTIII, and COS7/FTIV cells were harvested at 80% confluence and washed 4× with Hanks’ buffered saline solution (HBSS). The cells were suspended in solubilization buffer and sonicated by three, 30 s bursts on a Branson Sonifier at 4°C. The homogenates were centrifuged at 14,000 r.p.m. for 30 min at 4°C. The supernatants were recovered and used directly for assays, or aliquots were prepared and stored at -80°C.

Antibodies

Anti-Lea (CD-15) and anti-sLea (CSLEX-1) mAbs were obtained from Becton Dickinson (San Jose, CA). Anti-Leb (BG 5), anti-Leb (BG 6), anti-Leb (BG 8) and blood group H (BG4) mAbs were obtained from Signet (Dedham, MA). Goat anti-mouse IgM-peroxidase, goat anti-mouse IgG-peroxidase (γ-chain-specific) were obtained from Kirkegaard and Perry (Gaithersburg, MD). Anti-hamster IgG mAb (biotin-conjugated) was purchased from Pharmingen, (San Diego, CA). Goat anti-mouse IgG/IgM-peroxidase, goat anti-mouse IgG-peroxidase (1:5000), and reactive wells were blocked by incubation with 5% BSA in PBS at 37°C for 2 h. The wells were washed and incubated with 100 µl of 10 µg/ml solutions of mAb to Lea, sLea, Leb, Leb, and blood group H in dilution buffer at room temperature for 1 h. The wells were washed 4× with PBS-Tween (0.05% Tween 20) and incubated at room temperature for 1 h with 100 µl of 1:500 dilution of goat anti-mouse IgG/IgM-peroxidase conjugate. The wells were washed 4× with PBS-Tween, followed by two washes with deionized water and incubated at room temperature for 2 h with 100 ml of ABTS-peroxidase purchased from Kirkegaard and Perry (Gaithersburg, MD). The absorbance of the wells was measured at 405 nm on a microtiter plate reader (Molecular Devices, Sunnydale, CA).

ELISA

ELISA for detection of Lea and other fucose-containing antigens was performed using buffers and procedures described previously (Nyame et al., 1996, 1997). Microtiter plates (96 wells) were coated overnight, at room temperature with 100 µl of 10 µg/ml solution of parasite extracts in coating buffer and subsequently blocked by incubation with 5% BSA in PBS at 37°C for 2 h. The wells were washed and incubated with 100 µl of 10 µg/ml solutions of mAb to Lea, sLea, Leb, Lea, and 100 µl of serially diluted sera from infected rodents was carried out essentially as described previously (Nyame et al., 1997). The neoglycoproteins prepared from bovine serum albumin, LNFPII-BSA, LNnT-BSA, LNFPIII-BSA, and sLea-BSA were purchased from V-Labs Inc. (Covington, LA). Microtiter plates were coated with 50 µl of 5 µg/ml LNFPIII-BSA, LNFPII-BSA, LNnT-BSA or sLea-BSA, blocked with BSA, and incubated with 50 µl of serially diluted sera from infected rodents. The wells were subsequently incubated with goat anti-mouse IgM-peroxidase (1:10,000) or goat anti-mouse IgG-peroxidase (1:5000), and reactive wells were detected by incubation with ABTS-peroxidase substrate and absorbance measurements at 405 nm on a microtiter plate reader. For detection of bound hamster IgG, the wells were incubated with mAb to hamster IgG (1 µg/ml) for 1 h before incubation with goat anti-mouse IgG-peroxidase.

Western blot

Approximately, 30 mg of parasite or cell extracts were analyzed by SDS–PAGE on 10% acrylamide gel under reducing conditions and transferred onto nitrocellulose filter. The filter was blocked by incubation with 5% solution of nonfat dry milk in TBS (10 mM Tris, 150 mM NaCl, pH 7.5) for 1 h at room temperature. The filter was washed 4× with TBS-Tween (10 mM Tris, 300 mM NaCl, pH 7.5, 0.05% Tween) and incubated with 10 µg/ml solution of anti-Lea mAb in dilution buffer (10 mM Tris, 300 mM NaCl, pH 7.5, 1% BSA, 0.3% Tween-20) for 1 h at room temperature. The filter was washed once again (4×) and incubated with 1:500 dilution of goat anti-mouse IgM-peroxidase conjugate for 1 h at room temperature. The filter was washed (4×) and bound.
antibodies were detected by a 30 s incubation with Enhanced Chemiluminescence (ECL) reagent (Amersham, Arlington Heights, IL) and autoradiography.

**Lectin blots**
Total detergent extracts of parasites or cells (~3 mg) were preabsorbed on streptavidin-Sepharose to remove endogenous biotin-containing proteins and then fractionated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked by incubation with 3% BSA solution in TBS (10 mM Tris, 150 mM NaCl, pH 7.5), washed 4× in TTBS-2 (10 mM Tris, 0.5 M NaCl, 0.3% Tween-20), and incubated in 1 μg/ml solution of biotinylated WFA or LTA in 10 mM Tris, 150 mM NaCl, 0.05% Tween-20, and 1% BSA, with or without 200 μM GalNAc for the WFA and 500 mM fucose for LTA treatments, respectively. The membranes were washed 4× with TTBS-2 and incubated with 1:5000 dilution of streptavidin–peroxidase (Boehringer-Mannheim, Indianapolis, IN) in 10 mM Tris, 150 mM NaCl, 0.05% Tween-20 and 1% BSA. The membranes were washed with TTBS-2 for 4× times. Incubations were carried out at room temperature for 1 h, and the washes were done for 10 min per each wash. Reactive bands were visualized by the ECL technique.

**Flow cytometry**
COS7 and COS7/FTIV cells were harvested at 80% confluent with 1 ml HBSS/NGS and resuspended in 100 μl of Pooled human sera diluted 1:20 in HBSS containing 10% NGS (HBSS/NGS). The cells were washed 4× with 1 ml HBSS/NGS and resuspended in 100 μl 1:50 dilution of goat anti-human IgM-FITC conjugate and incubated for 30 min at 4°C. The cells were washed again (4×) with HBSS/NGS and bound antibodies were analyzed on a FACStar flow cytometer (Becton Dickinson, San Jose, CA).

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