Rodents infected with *Schistosoma mansoni* produce cytolytic IgG and IgM antibodies to the Lewis x antigen


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*S. mansoni* is a blood fluke that produces glycoconjugates containing the Lewis x antigen (Le*) Galβ1→4(Fucα1→3) GlcNAcβ1→R. However, Le* antigen is also normally expressed in many tissues of adult rodents. We now report that mice and hamsters chronically infected with *S. mansoni* generate high titers of both IgM and IgG antibodies reactive with Le* and that no reactivity is present in sera from uninfected animals. Anti-Le* antibodies were detected by ELISA using the Le*-containing neoglycoprotein lacto-N-fucopentaoselll-BSA. The IgG in infected animals consists of IgG1, IgG2a, and IgG2b subclasses and binds to Protein A-Sepharose. The sera of infected animals react only with Le* antigen and has no reactivity toward either Le* or sialyl Le*. The IgM response to Le* is detectable at week 2, whereas the IgG response is detectable at weeks 5-6 following infection of mice. The sera of infected mice and hamsters can mediate the complement-dependent cytosis (CDC) of cells expressing surface Le*. This cytolytic activity is exclusively effected by the anti-Le* antibodies, since their removal from sera by adsorption depletes the sera of CDC activity. Thus, the abundant expression of the Le* antigens by the parasite elicits cytolytic antibodies reactive with a host antigen.

**Key words:** *S. mansoni* / rodent / IgM / IgG / Lewis x

Introduction

*Schistosoma mansoni* synthesizes a number of glycoproteins containing complex-type tri- and tetraantennary N-glycans with outer branches having the repeating disaccharide (3Galβ1→4GlcNAcβ1→)ₙ or polylactosamine sequence (Srivatsan et al., 1992; Cummings and Nyame, 1996). Many of the glycoproteins also carry the Lewis x determinant (Le*) Galβ1→4(Fucα1→3)GlcNAcβ1→R in their outer chains and within the polylactosamine sequences (pLe*) (Srivatsan et al., 1992). The pLe* structure also occurs in O-glycans on the surface of both adult schistosomes and schistosomula, but Le* is not detectable in cercariae and miracidia (Koster and Strand, 1994). Schistosomes lack siaic acid and do not express sialylated glycoconjugates, such as the sialyl Le* antigen (Srivatsan et al., 1992).

The Le* antigen is normally expressed in many peripheral tissues in rodents, particularly the urogenital organs and cerebellum (Fox et al., 1981). Le* antigen, also called the stage-specific embryonic antigen-1 or SSEA-1, was among the earliest described differentiation antigens identified in mice (Solter and Knowles, 1978; Fox et al., 1981; Gooi et al., 1981; Lagenaur et al., 1982). The gene encoding an α1,3-fucosyltransferase capable of synthesizing the Le* antigen is expressed in many rodent tissues, such as epithelial tissues and bone marrow (Gersten et al., 1995). Interestingly, mice mount a vigorous IgM response to Le* antigens, when immunized with cells expressing the antigen, but no IgG response has been documented (Solter and Knowles, 1978; Fox et al., 1981; Huang et al., 1983).

We recently discovered that sera from humans and primates infected with *S. mansoni* contain anti-Le* antibodies that can mediate the complement-dependent cytosis (CDC) of human leukocytes expressing the Le* antigen (Nyame et al., 1996). Because rodents are used as a major model for studying schistosomiasis, we have now conducted a systematic examination of sera from infected mice and hamsters for the presence of anti-Le* and for the isotypes of antibodies produced. Our results demonstrate that the sera of infected animals contain very high titers of both IgG and IgM toward Le* and that these antibodies can mediate the CDC of target cells expressing the Le* determinant. These findings raise many questions about the role of the Le* determinant in schistosome survival in infected animals and suggest the possibility that autoimmune complications could arise from the strong response to Le* antigens.

**Results**

**Presence of anti-Le* antibodies in sera from infected animals**

The induction of antibody responses in rodents to the Le* determinant on schistosomes was investigated using five mice and five hamsters chronically infected with *S. mansoni*. Sera from five uninfected mice and five uninfected hamsters were analyzed as controls. The presence of anti-Le* antibodies in the sera of the infected rodents was detected by ELISA using LNFPIII-BSA as the target antigen. LNFPIII is a fucosylated pentasaccharide Galβ1→4(Fucα1→3)GlcNAcβ1→3Galβ1→4Glc containing a single Le* determinant.

Sera from each of the infected mice contained both IgM and IgG antibodies that bound to LNFPIII-BSA and the reactivity could be detected at 1:1600 dilution of sera (Figure 1). Sera from uninfected mice did not bind to LNFPIII-BSA (Figure 1). Sera from infected hamsters also contained both IgM and IgG antibodies reactive to LNFPIII-BSA and the reactivity could
Fig. 1. Presence of anti-Le* in the sera of infected mice. Microtiter wells were coated with LNFPIII-BSA and incubated with sera from different mice serially diluted 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200, 1:6400. Bound antibodies were detected by incubation with alkaline phosphatase labeled goat anti-mouse IgM or IgG, as described in Materials and methods. Each plot is an average of triplicate assays and represents analysis of serum from an individual mouse. (A) Infected mice IgM antibody response; (B) uninfected mice IgM antibody response; (C) infected mice IgG antibody response; (D) uninfected mice IgG response.

similarly be detected up to 1:1600 dilution of sera. Sera from uninfected hamsters tested negative for LNFPIII-BSA binding (Figure 2). As a control, no antibody binding was observed for either the infected or uninfected mice or hamsters when underivated BSA was used as a target (data not shown).

To confirm the specificity of the antibodies, we tested their reactivity with LNnT-BSA. LNnT is a tetrasaccharide Galβ1→4GlcNAcβ1→3Galβ1→4Glc lacking the fucose residue found in LNFPIII. Neither IgM nor IgG antibodies in the sera of the infected animals bound to LNnT-BSA (Figure 3). These results confirm that the fucosyl residue in the Le* structure is critical for recognition by the anti-Le* antibodies in the sera of infected animals. The sera from the infected animals were tested further for reactivity toward other Lewis antigens. Pooled sera from 8-week infected mice were analyzed for reactivity to Le* and sialyl Le* targets. No antibody reactivity toward these antigens was detectable (Figure 4). These results demonstrate that the antibody to Le* antigen in the sera of infected animals is highly specific and does not simply recognize fucose moieties.

Characterization of the IgG response to Le* antigen in infected animals

Because an IgG response to Le* antigens has not been clearly documented in previous studies, we performed additional experiments to verify this finding. Pooled sera from infected or uninfected hamsters was passed over a column of Protein A-Sepharose. Bound antibodies were eluted and tested for reactivity to LNFPIII-BSA targets. A strong response was found for the IgG purified from infected animals, but the IgG from uninfected animals failed to react with the target (Figure 5). Taken together, the results demonstrate conclusively that re-
Fig. 2. Presence of anti-Le<sup>x</sup> in the sera of infected hamsters. Serially diluted hamster sera were applied to LNFPII-BSA coated microtiter wells as described in Figure 1. Bound IgM antibodies were detected with goat anti-mouse IgM-alkaline phosphatase conjugate, and IgG antibodies were detected by incubation with goat anti-hamster IgG antibodies, followed by incubation with rabbit anti-goat IgG-alkaline phosphatase conjugate, as described in Materials and methods. Each plot is an average of triplicates and represents analysis of serum from individual hamster. (A) Infected hamster IgM antibody response; (B) uninfected hamster IgM antibody response; (C) infected hamster IgG antibody response; (D) uninfected hamster IgG response.

Rats infected with <i>S.mansoni</i> mount an IgG response to the Le<sup>x</sup> antigen.

The subclass distribution of the IgG antibodies reacting with Le<sup>x</sup> determinants was characterized for the infected mice sera by ELISA using LNFPIII-BSA targets. Bound IgG subclasses were detected by anti-mouse IgG subclass antibodies. LNFPII binding IgG antibodies from all the five infected mice contained IgG1, IgG2a, and IgG2b subclasses (Figure 6). The presence of anti-Le<sup>x</sup> IgG2a and IgG2b indicates that the sera of the infected animals could mediate complement-dependent cytolyis of Le<sup>x</sup> bearing target cells.

**Longitudinal studies on infected mice**

To determine how long it takes for infected mice to mount an IgM and IgG response to the Le<sup>x</sup> antigen, we performed a longitudinal study. Ten Swiss Webster mice were infected with 250 cercariae and 10 uninfected mice served as controls. Blood was drawn from tails each week following infection and tested for reactivity with LNFPII-BSA in ELISA. The IgM response to the Le<sup>x</sup> antigen was detected by week 2 and peaked in a biphasic pattern at weeks 4 and 7 (Figure 7). In contrast, the IgG response to the Le<sup>x</sup> antigen was detected at weeks 5–6 and continued to increase thereafter. No anti-Le<sup>x</sup> response was detected at any time in the 10 uninfected control mice (data not shown).

**Analysis of sera from infected rodents for binding of antibodies to HL-60 cells**

The specificity of the anti-Le<sup>x</sup> antibodies was characterized further by investigating their reactivity towards Le<sup>x</sup> determinants on glycoconjugates present on cell surfaces. Antibodies in sera from the infected rodents were tested for their ability to bind cells expressing the Le<sup>x</sup> antigen. Human promyelocytic leukemia HL-60 cells express Le<sup>x</sup> and the related sialyl Le<sup>x</sup> determinants on their surfaces (Fukuda et al., 1984; Fukushima et al., 1984; Kumar et al., 1991). HL-60 cells incubated with
sera from infected mice and hamsters were stained intensely, but no staining was observed for HL-60 cells incubated with sera from uninfected mice or hamsters (Figure 8). These results, together with data from ELISA, show that the antibodies in the sera of the infected animals react specifically with Le\(^x\) determinants and that the reactivity is unaffected by the backbone on which the Le\(^x\) determinant is presented.

**Complement-dependent cytolysis of HL-60 cells mediated by sera from infected rodents**

The high titer and isotypes of antibodies to Le\(^x\) in infected animals led us to consider whether these antibodies could mediate complement-dependent cytolysis of cells expressing Le\(^x\) determinants. Pooled sera from infected mice or hamsters were incubated with HL-60 cells in the presence or absence of complement and monitored for cytolysis by propidium iodide uptake. Sera from infected mice and hamsters caused cytolysis of HL-60 cells in a complement-dependent fashion, whereas sera from uninfected mice and hamsters did not mediate HL-60 cell lysis either in the presence or absence of complement (Figure 9).

To determine the extent to which anti-Le\(^x\) antibodies contributed to the complement-dependent cytolysis of HL-60 cells, sera from infected hamsters were depleted of the anti-Le\(^x\) antibodies and then tested for cytolytic activity. Depletion of anti-Le\(^x\) antibodies was accomplished by absorbing the sera with COS7 cells generated to stably express the human \(\alpha1,3\)-fucosyltransferase IV (FTTV). This enzyme is capable of synthesizing the Le\(^x\) and sialyl Le\(^x\) antigen (Goelz et al., 1990; Kumar et al., 1991; Lowe et al., 1991). However, expression of FTV in COS7 cells causes surface expression of Le\(^x\), but not sialyl Le\(^x\) (Lowe et al., 1991). As controls, wild type COS7 cells, which lack any \(\alpha1,3\)-fucosyltransferase and are incapable of synthesizing Le\(^x\) antigens, were also used in absorption. Analysis of the absorbed sera by ELISA using LNFPIII-BSA targets showed that the sera absorbed with COS7/FTTV cells...
Antibodies to Lewis x in *S. mansoni* infected rodents

Fig. 4. Reactivity of sera in infected mice toward different Lewis antigens. Serially diluted pooled sera from 8-week infected Swiss Webster mice were analyzed by ELISA for the presence of (top) IgM or (bottom) IgG antibodies to LNFPD-BSA (●), LNFPDI-BSA (△), sialyl Le^x^-BSA (□), or LNnT-BSA (○). The ELISA utilized either goat anti-mouse IgM-peroxidase or goat anti-mouse IgG-peroxidase, as described in Materials and methods.

had no anti-Le^x^ reactivity, whereas the sera absorbed with wild type COS7 cells retained full anti-Le^x^ reactivity (Figure 10). This loss of reactivity in sera absorbed against COS7/FTTV cells was specific for the Le^x^ antigen, since the sera still retained significant reactivity with antigens in total detergent extracts of *S. mansoni* (Figure 10).

Sera from infected hamsters absorbed against wild-type COS7 cells mediated complement-dependent cytolysis of HL-60 cells, but infected hamster sera absorbed on COS7/FTTV had no complement-dependent cytolytic activity (Figure 11). These results demonstrate that anti-Le^x^ antibodies in the sera of *S. mansoni* infected mice and hamsters are complement fixing and that they mediate lysis of target cells bearing surface Le^x^ determinants. Furthermore, these results demonstrate that the major cytolytic activity of the sera from infected animals toward cells is due to the anti-Le^x^ reactivity.

Discussion

These studies have demonstrated the presence of both IgG and IgM antibodies to Le^x^ antigens in mice and hamsters infected

Fig. 5. Binding of IgG antibodies purified by Protein A-Sepharose affinity chromatography to LNFPDI-BSA. LNFPDI-BSA targets were incubated in triplicate with either 100 μg/ml, 100 μg/ml, 200 μg/ml solution of IgG purified from infected (●) or uninfected (○) pooled hamster sera by chromatography on Protein A-Sepharose or with 100 μl of 1:500 dilution of goat anti-hamster IgG-alkaline phosphatase conjugate using ELISA procedures described in Materials and methods.

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Fig. 6. Analysis of the subclasses of anti-Le^x^ IgG antibodies in infected mouse sera by ELISA. Sera from *S. mansoni* infected CD1 mice were diluted 1:100 and incubated with LNFPDI-BSA targets. Bound IgG subclass antibodies were detected using goat anti-mouse IgG1, IgG2a, and IgG2b antibodies and ELISA procedures described in Materials and methods.
could be due to unique presentation of the Le* determinants and/or the dose (worm burden) of membrane-bound or secreted antigens were detected, indicating that the anti-Le* antibodies are highly specific in their recognition of antigen. The anti-Le* antibodies can mediate the complement-dependent cytosis of cells expressing the Le* determinant. More importantly, the anti-Le* antibodies in sera from infected animals are primarily responsible for the observed cytolytic activity. In complementary studies we recently found that the sera of humans and rhesus monkeys infected with *S.mansoni* contain IgM and IgG antibodies against the Le* antigen (Nyame et al., 1996). In *S.mansoni* infected Rhesus monkeys the titers of both IgM and IgG antibody to Le* antigens were comparable to those observed in infected rodents, whereas the titers in humans were lower and were primarily IgM. This lower level observed in these preliminary studies in infected humans may reflect a lower worm burden or other unknown factors. The documented presence of antibodies to the Le* antigen in infected rodents and primates makes the rodent model system useful for studying host responses to the Le* antigen.

Antibodies previously reported to be reactive to Le* antigens are exclusively of the IgM isotype (Solter and Knowles, 1978; Huang et al., 1983). The production of IgG anti-Le* antibodies in infected rodents may result from the persistent presentation and/or the dose (worm burden) of membrane-bound or secreted Le* antigens. The circulating cathodic antigen (CCA) secreted from the female schistosome gut cells contains Le* antigens on O-glycans and the concentration of CCA in serum is relatively high (van Dam et al., 1994) and may contribute to the immune response. Alternatively, production of IgG anti-Le* antibodies could be due to unique presentation of the Le* determinants within the parasite-derived glycoconjugates. Interestingly, the first phase of mouse IgM response to Le* antigen appears before the onset of egg laying or secretion of CCA, indicating that the host response to Le* determinants is in response to the developing schistosomula. Egg laying commences around weeks 6-7 and CCA is detected in sera of infected Swiss mice beginning around week 4 (van Dam et al., 1996). The second phase IgM response and the IgG response, however, occur around the time of oviposition. Further studies using unisexual infection will be necessary to determine whether egg antigens are important in the second phase IgM response and the IgG response. The early appearance of anti-Le* may be useful in the early detection of infection.

The brain and peripheral tissues of rodents express Le* determinants (Fox et al., 1981; Lagenaur et al., 1982), but these determinants are not known to be presented on mouse leukocytes or other blood cells. At present there is no direct evidence that the anti-Le* antibodies in infected rodents contribute to or cause an autoimmune disease. However, it has been noted that neutrophil granulocyte inflammatory reactions in *S.mansoni* infected mice are significantly reduced (Santos-da Silva et al., 1988). Furthermore, chronic schistosomiasis in humans causes an inhibition of neutrophil granulocytosis (Borojevic et al., 1983). More studies are required to determine whether the anti-Le* antibodies are involved in these observed impairments of neutrophil function or pathogenesis of schistosomiasis (Cummings and Nyame, 1996).

![Graph 7: Longitudinal study of infected mice for the appearance of anti-Le* IgM and IgG responses. Ten Swiss Webster mice were infected with 250 cercariae each, bled weekly, and the sera was diluted 1:200 in dilution buffer for ELISA analysis. Anti-Le* antibodies were identified using LNPHI-BSA and peroxidase-conjugated anti-mouse IgM (●) and anti-mouse IgG (○), as described in Materials and methods. Sera from 10 uninfected mice served as controls. Results shown represent the average of the absorbance for the 10 infected animals. No anti-Le* reactivity was observed at any time in sera from the uninfected, control animals (data not shown).](https://academic.oup.com/glycob/article-abstract/7/2/207/598120)

*Fig. 7. Longitudinal study of infected mice for the appearance of anti-Le* IgM and IgG responses. Ten Swiss Webster mice were infected with 250 cercariae each, bled weekly, and the sera was diluted 1:200 in dilution buffer for ELISA analysis. Anti-Le* antibodies were identified using LNPHI-BSA and peroxidase-conjugated anti-mouse IgM (●) and anti-mouse IgG (○), as described in Materials and methods. Sera from 10 uninfected mice served as controls. Results shown represent the average of the absorbance for the 10 infected animals. No anti-Le* reactivity was observed at any time in sera from the uninfected, control animals (data not shown).*

![Graph 8: Flow cytometric analyses of HL-60 cells incubated in sera from infected and uninfected rodents. Pooled sera from mice and hamsters were diluted 1:50 and incubated with 5 x 10^6 HL-60 cells. Bound IgM antibodies were probed by incubation with goat anti-mouse IgM-FITC conjugate followed by analysis on a flow cytometer. Analysis performed with sera from infected animals are designated 2 and those performed with sera from uninfected animals are designated 1. (A) Analysis of HL-60 cells incubated in mouse sera; (B) analysis of HL-60 cells incubated in hamster sera.](https://academic.oup.com/glycob/article-abstract/7/2/207/598120)

*Fig. 8. Flow cytometric analyses of HL-60 cells incubated in sera from infected and uninfected rodents. Pooled sera from mice and hamsters were diluted 1:50 and incubated with 5 x 10^6 HL-60 cells. Bound IgM antibodies were probed by incubation with goat anti-mouse IgM-FITC conjugate followed by analysis on a flow cytometer. Analysis performed with sera from infected animals are designated 2 and those performed with sera from uninfected animals are designated 1. (A) Analysis of HL-60 cells incubated in mouse sera; (B) analysis of HL-60 cells incubated in hamster sera.*
Antibodies to Lewis x in S.mansoni infected rodents

Mouse

Hamster

CD

CD

O

COS7 absorbed sera

COS7/FTTV absorbed sera

LNFPIII-BSA S. mansoni extract

Fig. 10. Loss of Anti-Le\textsuperscript{*} reactivity in infected hamster sera following absorption against COS7/FTTV cells expressing Le\textsuperscript{*}. Pooled infected hamster sera was incubated with either COS7 or COS7/FTTV cells. The recovered antisera following absorption was tested in an ELISA for reactivity toward either LNFPIII-BSA or total detergent extract of S.mansonii, as described in Materials and methods.

Fig. 9. Flow cytometric analyses of rodent sera-mediated complement-dependent cytolysis of HL-60 cells. Pooled sera from mice and hamsters were diluted 1:20 and incubated with approximately $2 \times 10^5$ HL-60 cells in the presence or absence of guinea pig complement. Cytolysis was determined on a flow cytometer following propidium iodide uptake. Open profiles represent experiments performed in the absence of complement and the shaded profiles represent experiments performed in the presence of complement. (A) HL-60 cells incubated with uninfected mice sera; (B) HL-60 cells incubated with infected mice sera; (C) HL-60 cells incubated with uninfected hamster sera; (D) HL-60 cells incubated in infected hamster sera.

Fluorescence Intensity

The degree to which the anti-Le\textsuperscript{*} antibodies we have identified participate in humoral immunity to the parasite is unknown. It has been shown that monoclonal antibodies to Le\textsuperscript{*} bind to schistosomula (Ko et al., 1990; Koster and Strand, 1994). Whether the anti-Le\textsuperscript{*} antibodies mediate killing of schistosomula is under investigation. Sera from infected animals has been reported to confer passive protection against infections in naive animals when given before cercarial challenge (Sher et al., 1975). Furthermore, antibodies purified from infected human sera over immobilized schistosomula extracts can mediate CDC of schistosomula in vitro and also confer some degree of passive protection in mice (Iwo and LoVerde, 1989).

We recently found that S.japonicum and S.haematobium also present Le\textsuperscript{*} antigens on high molecular weight glycoproteins and that humans infected with these species also contain anti-Le\textsuperscript{*} in their sera (unpublished data). The roles of Le\textsuperscript{*} antigens on schistosome glycoconjugates are not known. It has been proposed that Le\textsuperscript{*}-containing glycans can effect a Th1 to Th2 switch in S.mansonii infected mice, thereby limiting the cellular immune response (Velupillai and Ham, 1994). Interestingly, Le\textsuperscript{*} antigens have also been found in the lipopolysaccharides of certain strains of Helicobacter pylori (Aspinall and Monteiro, 1996; Aspinall et al., 1996). There is indirect evidence that antibodies in individuals infected with H. pylori may be directed against the Le\textsuperscript{*} determinant (Negrini et al., 1991). Collectively, these results raise the interesting possibility that the synthesis of the Le\textsuperscript{*} antigen is a common theme among many infectious organisms. It is currently an open question as to why parasites synthesize this determinant and whether the anti-Le\textsuperscript{*} contributes to pathogenesis of the disease.
Materials and methods

**Materials**

LNFPIII, LNTn, LNFPI-BSA, and sialyl Le'-BSA were purchased from V- Labs Inc. (Covington, LA). Goat anti-mouse IgM-alkaline phosphatase, goat anti-mouse IgG-alkaline phosphatase, rabbit-anti-goat IgG-alkaline phosphatase, goat anti-mouse IgM-alkaline phosphatase, goat anti-mouse IgG-alkaline phosphatase, rabbit-anti-goat IgG-alkaline phosphatase, goat anti-mouse IgM-alkaline phosphatase, goat anti-mouse IgG-alkaline phosphatase, and rabbit-anti-goat IgG-alkaline phosphatase were purchased from Boehringer-Manheim. Goat anti-hamster IgG-alkaline phosphatase, goat anti-mouse IgM-peroxidase, goat anti-mouse IgG-peroxidase and the peroxidase substrate ABTS were obtained from Kirkegaard and Perry Laboratories Inc. (Gaithersburg, MD). LNFPIII-BSA and LNTn-BSA were synthesized by the reductive amination method, as described previously (Nyame et al., 1996) using the method of Gray (1974).

**Sera and cell culture**

Unless otherwise indicated, infected mouse and hamster sera were prepared from CD1 mice and LVG hamsters infected for 7 wk with 150 and 400 S. mansoni cercariae (Puerto Rico strain), respectively. Infections were carried out at the University of Lowell in Lowell, MA, and infected animals, and uninfected control animals were shipped to our laboratory within 24 h. Normal goat serum (NGS) was kindly donated by Dr. Kevin Moore (Oklahoma Medical Research Foundation). HL-60 cells, wild type COS7 cells, and COS7/FTTV were cultured as described previously (Nyame et al., 1996).

**ELISA**

Microtiter wells (96 wells) were coated with 50 µl of 5 µg/ml solution of LNFPIII-BSA, LNTn-BSA, nialy Le'-BSA, or LNFPPII-BSA in coating buffer (150 mM Na2CO3, 348 mM NaHCO3, 0.02% NaN, pH 9.3) at 37°C for 2 h. In some experiments microtiter plates were coated with 20 µg/ml of the total detergent extract of S. mansoni. The wells were washed six times with PBS (6.7 mM KH2PO4, 150 mM NaCl, 0.02% NaN, pH 7.4) and subsequently blocked with 300 µl 5% BSA in PBS for 2 h at 37°C. The wells were washed six times with PBS–TWEEN-20 (PBS containing 0.5% Tween-20) and incubated overnight at 4°C with 50 µl of sera diluted serially in dilution buffer (PBS containing 1% BSA and 0.3% TWEEN-20). Serum dilutions used were 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200, and 1:6400. The wells were washed six times with PBS–TWEEN-20 and incubated for 1 h at room temperature with 50 µl of goat anti-mouse IgM– or IgG-alkaline phosphatase conjugate diluted 1:1000 in dilution buffer. The wells were washed six times with PBS–TWEEN-20, followed by two washes with deionized water and incubation with 100 µl of substrate solution (1 mg/ml p-nitrophenyl phosphate, 1 mM NaCl, 50 mM Na2HPO4, 150 mM NaCl, 10 mM Na2HPO4, pH 8.4) and applied to 2 ml columns of Protein A-Sepharose pre-equilibrated in borate buffer. The columns were rotated for 2 h at room temperature and washed with 10 column volumes of borate buffer followed by a wash with 1 M NaCl, 10 mM Na2HPO4 (pH 8.4) until no protein could be detected by absorbance at 280 nm. The bound material was eluted sequentially with 1 ml of 100 mM glycine, pH 3.0, and neutralized with 100 µl 1 M Tris, pH 8.0. Fractions containing protein were pooled and dialyzed against PBS, and protein concentration was determined by BCA protein assay. The samples were stabilized by adding 4 mg/ml BSA and used immediately or aliquoted and stored at −80°C.

**Purification of hamster IgG by chromatography on Protein A-Sepharose**

Pooled sera from infected or uninfected hamsters (2 ml) were adjusted to 1 M NaCl, 50 mM Na2HPO4, pH 8.4 (borate buffer) with 2 M NaCl, 100 mM Na2HPO4 (pH 8.4) and applied to 2 ml columns of Protein A-Sepharose pre-equilibrated in borate buffer. The columns were rotated for 2 h at room temperature and washed with 10 column volumes of borate buffer followed by a wash with 1 M NaCl, 10 mM Na2HPO4 (pH 8.4) until no protein could be detected by absorbance at 280 nm. The bound material was eluted sequentially with 1 ml of 100 mM glycine, pH 3.0, and neutralized with 100 µl 1 M Tris, pH 8.0. Fractions containing protein were pooled and dialyzed against PBS, and protein concentration was determined by BCA protein assay. The samples were stabilized by adding 4 mg/ml BSA and used immediately or aliquoted and stored at −80°C.

**Staining of HL-60 cells with sera**

HL-60 cells were harvested at 80% confluence and washed four times with HBSS, and 5 x 10³ cells were incubated with 100 µl of 1:50 dilution of mouse or hamster sera in 5% NGS/HBSS for 30 min at 4°C. The cells were washed four times with HBSS and incubated with 100 µl of 1:50 dilution of goat anti-mouse IgM–FITC (Boehringer-Manheim) in 5% NGS/HBSS for 30 min at 4°C. The cells were washed four times with HBSS and assessed for antibody binding by analysis in a FACStar flow cytometer (Becton Dickinson, San Jose, CA).

**Complement-dependent cytotoxicity of HL-60 cells**

HL-60 cells were harvested at 80% confluence and washed four times with HBSS, and their viability was determined by trypan blue staining. Approximately 2 x 10³ cells were incubated in duplicate with 200 µl of 1:50 dilution of sera in 5% NGS/HBSS at 37°C for 1 h. Guinea pig complement was added to one set of cells to a final dilution of 1:20 in a total volume of 250 µl. The viability of the other set of cells was adjusted to 250 µl with 5% NGS/HBSS. All the cells were incubated further for 1 h at 37°C. Finally, propidium iodide was added to a final concentration of 20 µg/ml and the cells were analyzed by flow cytometry. Sera used in the assays were preincubated at 57°C for 30 min to inactivate endogenous complement.

**Absorption of sera with COS7 and COS7/FTTV cells**

COS7 and COS7/FTTV cells were harvested at 80% confluence and washed four times with HBSS. Approximately 0.1 ml of packed cells were incubated with 200 µl of serum for 4 h at 4°C, and the serum was recovered from cells by centrifugation at 1000 r.p.m. for 5 min.

**Preparation of total detergent extracts of adult S. mansoni**

Adult schistosomes were recovered from infected hamsters and placed in solubilization buffer, which consisted of PBS containing 0.1% Triton X-100 and the following protease inhibitors: EDTA (37 µg/ml), trypsin inhibitor (10 µg/ml), PMSE (1 mM), aprotinin (2 µg/ml), leupeptin (0.7 µg/ml), and 3,4-dichloroisocoumarin (200 µM). The worms were then sonicated on a Branson Sonifier at 4°C using three 30 s bursts. The sonicates were incubated on ice for 30 min and then centrifuged at 3000 x g for 30 min at 4°C to remove large debris. The supernatants were removed and centrifuged further at 100,000 x g for 1 h at 4°C. The resulting supernatants were recovered and total protein determined by the BCA protein assay. These total detergent extracts were used directly or aliquoted and stored at −80°C until use.

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**Abbreviations**

Le³, Lewis x, GaLb1→4(Fucα1→3)GalNAc-R; LNFPIII, lacto-N-fucopentaose III GaLb1→4(Fucα1→3)GalNAcβ1→3Galβ1→4Glc; LNFPPII, lacto-N-fucopentaose GaLb1→3(Fucα1→4GlcNAcβ1→3Galβ1→4Glc; LNIT, lacto-N-neotetraose GaLb1→4GlcNAcβ1→3Galβ1→4Glc; BSA, bo-

**Longitudinal studies on anti-Le³ expression in infected mice**

In longitudinal studies on infected mice, 10 Swiss Webster mice were infected with 250 cercariae at the Biomedical Research Institute (Rockville, MD) and shipped to our laboratory within 24 h. Ten, age-matched uninfected Swiss Webster mice of the same sex were purchased from Taconic (Germantown, NY) and served as controls. Blood was collected at weekly intervals from the tails and was diluted 1:200 in dilution buffer for ELISA analysis. The anti-Le³ reactivity was monitored as described above, except that goat anti-mouse IgM-peroxidase and goat anti-mouse IgG-peroxidase (1:5000 dilution) were used instead of the alkaline phosphatase conjugates. Reactivity was determined toward either immobilized LNFPIII-BSA or BSA.
Antibodies to Lewis x in S.mansoni infected rodents

References


