PLACENTAL AND ORAL DELIVERY OF SCHISTOSOMA MANSONI ANTIGEN FROM INFECTED MOTHERS TO THEIR NEWBORNS AND CHILDREN

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Abstract. A 63-kD Schistosoma mansoni antigen was detected in 149 (86%) of 174 umbilical cord blood sera from infected women at delivery. Specific IgG antibodies to this antigen were also detected in 80% of cord blood sera. The 63-kD antigen showed the same molecular mass by Western blotting when isolated from cord blood, maternal blood, breast milk, and urine from women infected with S. mansoni. This antigen was detected in the urine of 25 infants born to infected mothers and followed for 18–24 months after delivery. It was also detected in some infants up to 21 days after parturition and then disappeared at 28 days, demonstrating the influence of breast-feeding on persistence of antigen in infants born to infected women. Thus, exposure to Schistosoma antigens and maternal antibodies to this organism may influence the developing immune responses to natural infection or vaccination of children born in endemic areas.

INTRODUCTION

The major challenge in the development of vaccines against infections with Schistosoma is to use defined antigens to stimulate an appropriate immune response that leads to resistance. However, the vaccines developed may not produce satisfactory seroconversion in children, who still have maternal antibodies for specific vaccine antigens. Inhibition of vaccine responses by maternal antibodies was initially observed with live measles and oral poliomyelitis vaccines, as well as with inactivated pertussis, tetanus, and diphtheria toxoid vaccines, and recently, hepatitis A virus vaccine. Exposure to maternal schistosome antigens might also predispose a child to respond with an altered, perhaps more immunoregulated form of schistosomiasis. Thus, defining the extent to which maternal schistosome antigens may or not interfere with responses to new infant vaccines requires a better understanding of its main determinants.

We have previously identified a 63-kD antigen in different extracts of Schistosoma mansoni cercariae, adult worms, and eggs and in the urine of S. mansoni-infected individuals by an IgG2a-specific monoclonal antibody (MAb). In the present study, we have evaluated both the placental and oral delivery of the 63-kD S. mansoni antigen from infected mothers to their newborns.

MATERIALS AND METHODS

Samples. Blood and urine samples were collected from 180 pregnant women 18–36 years of age (mean ± SD = 25.626 ± 4.733, median = 25) who had S. mansoni eggs in their stool, and from 25 uninfected pregnant women 19–30 years of age (mean ± SD = 24.5 ± 3.94, median = 25) who did not have parasite eggs in their stool. Samples were obtained at the Gynecology and Obstetrics Department of the Mansoura University Hospitals in Mansoura, Egypt. The uninfected women were from the same endemic area (Dakahlia governorate, Egypt) and had negative serology to schistosome antigens when tested by an enzyme-linked immunosorbent assay (ELISA). Simple stool sedimentation analysis was done for two or three consecutive days for each woman. A Kato thick smear technique was done for 47 infected women: 25 women with mild infections (<100 eggs per gram [egp] of feces), 18 with moderate infections (101–400 egp), and 4 with heavy infections (>400 egp). Cord blood samples were collected at delivery from all 205 women (i.e., 180 infected and 25 uninfected). Blood and cord blood sera were separated and stored at −70°C until used. To avoid using cord blood contaminated with maternal blood at delivery, we routinely tested for presence of IgM in cord blood samples using an ELISA described later in this report. Cord blood serology detected IgM in six of 205 cord blood serum samples, confirming cross-contamination of these six cord sera with maternal IgM at delivery. These six cord blood serum samples were from infected mothers and were excluded from the study. Informed consent was obtained from all participants, and they were fully informed concerning the diagnostic procedures involved and the nature of the disease. A full clinical history was obtained from all mothers with special emphasis on symptoms attributed to infection with S. mansoni and treatment of this infection or its complications. Complete general and systemic examinations were performed for all mothers, with special emphasis on the size and tenderness of the liver, spleen, and kidneys. Most of infected women reported a history of schistosome infection and some were previously treated at least two times before their current pregnancy. The study was reviewed and approved by the Ethical Committee of the Mansoura University Hospitals.

Detection of total IgM in cord blood serum samples. After optimization of reaction conditions, polystyrene, flat-bottom, microtiter plates were coated with 50 µL/well of cord blood serum diluted 1:100 in carbonate buffer, pH 9.6. After blocking with 0.3% nonfat milk, 50 µL/well of anti-human IgM alkaline phosphatase conjugate (Sigma, St. Louis, MO) diluted 1:750 in phosphate-buffered saline (PBS), pH 7.2, containing 0.05% Tween 20 (PBS-T20) was added and the plates were incubated for one hour at 37°C. Substrate (1 mg/mL of p-nitrophenyl phosphate; Sigma) was then added, and the absorbance was read at 405 nm using a Σ960 microplate autoreader (Axiom, Burstadt, Germany). The cut-off optical density (OD) value for ELISA positivity (0.055) was the mean OD plus three standard deviation values for 10 cord blood sera.

Samples collected during the breast-feeding period and after parturition. Twenty-five infants born to S. mansoni-infected mothers and five infants born to uninfected mothers served as negative controls and were followed during the breast-feeding period (18–24 months) and for four weeks after parturition. Urine, serum, and breast milk samples were collected from their mothers. Only urine samples were col-
lected from the 30 newborns during the breast-feeding period. After parturition of the 30 newborns, a urine sample was collected weekly from each child for one month. During these periods, all infants were not exposed to sources of *Schistosoma* infection. Complete clinical examinations were performed for all infants, and none showed *Schistosoma* eggs in their stool during the study.

**Schistosoma mansoni soluble egg antigen.** Soluble egg antigen (SEA) was prepared according to the method of da Silva and Ferri.9 The protein content was determined using the Lowry method.10 The antigenic extract was stored at −70°C until use.

**Sodium dodecyl sulfate–polyacrylamide gel electrophoresis.** Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 0.75 mm-thick, 16% vertical slab gels according to the method of Laemmli.11 Serial concentrations of the tested samples (SEA, maternal blood serum, cord blood serum, urine, and breast milk) were mixed with the sample buffer (0.125 M Tris base, 4% SDS, 20% glycerol, 10% β-mercaptoethanol, and 0.1% bromophenol blue as a tracking dye) and immediately boiled for three minutes. A mixture of reference proteins (Promega, Madison, WI) was run in parallel. Gels were then stained with Coomassie blue or silver.

**Gel electroelution.** The band of interest (63 kD) was cut from preparative polyacrylamide gels and electroeluted from the gels in a dialysis bag (Sigma) as described by Attallah and others.6 The protein content of the purified antigen was then determined10 and the antigen was stored at −70°C until use.

**Western blotting.** Resolved samples separated by SDS-PAGE were electro-transferred12 onto nitrocellulose membranes (0.45 μm pore size). Blotting was carried out with a constant voltage of 60 volts for two hours. The immunoblot was then performed using an IgG2a MAb (C5C4), followed by incubation for two hours with goat anti-mouse IgG alkaline phosphatase conjugate (Sigma).5 The nitrocellulose membrane was then soaked in premixed alkaline phosphatase substrate (5-bromo-4-chloro-3-indolyl phosphate [BCIP], nitro blue tetrazolium [NBT], and 0.1 M Tris buffer, pH 9.6; ABC Diagnostics, New Damietta, Egypt). The color was observed within 10 minutes and the reaction was stopped by the addition of distilled water.

**Measurement of human schistosome-specific antibody response using an ELISA.** The specific IgG and IgM antibodies against the purified 63-kD *Schistosoma* antigen and SEA were evaluated in maternal blood serum and cord blood serum using the indirect ELISA described by Attallah and others.7 Briefly, flat-bottom, polystyrene, microtiter plates (Corning Life Sciences, Acton, MA) were coated with 2.5 μg/well of 63-kD antigen or 1.25 μg/well of SEA. After blocking with 0.3% nonfat milk, maternal and cord serum samples diluted 1:200 in PBS-T20 were added (50 μL/well), and the plates were incubated for two hours at 37°C. The plates were then washed five times with PBS-T20, anti-human IgG (or IgM) alkaline phosphatase conjugate (The Binding Site, Birmingham, United Kingdom) diluted 1:500 was added and incubated for one hour at 37°C. The plates were then washed again and substrate (p-nitrophenyl phosphate in 0.1M glycine buffer, pH 10.4) was added and the plates were incubated for 20 minutes at 37°C. The ODs were read at 405 nm using a Σ960 microplate autoreader. The maternal and cord blood sera of 10 uninfected women were used to set the cutoff limit of the ELISA. The cut-off OD for IgG ELISA positivity to 63-kD antigen was 0.235 (the mean OD plus three standard deviation values for samples from uninfected women). The cut-off OD was 0.351 for IgG ELISA positivity to SEA and 0.158 for IgM-ELISA positivity to SEA.

**Dot-ELISA.** A dot-ELISA,6,7 a sensitive and specific assay for qualitative detection of *Schistosoma* antigens in urine, was adapted to detect the reactivity of the 63-kD *Schistosoma* circulating antigen in blood serum and cord blood serum. All the assay steps were carried out on the surface of nitrocellulose membranes fixed in plastic device. Two hundred microliters of serum sample diluted 1:10 in PBS, pH 7.2, were added per dot. Blocking of the non-specific binding sites on the nitrocellulose membrane was done with 5% bovine serum albumin (Sigma) in PBS. The C5C4 mAb was then added at a dilution of 1:300 in PBS. After the samples were washed three times with PBS, anti-mouse IgG alkaline phosphatase conjugate (Sigma) at a dilution of 1:500 in PBS was added. The color was developed after addition of alkaline phosphatase premixed BCIP/NBT substrate system (ABC Diagnostics) for two minutes. The reaction was then stopped with distilled water and the result was recorded. The purple color produced in the dot-ELISA for a positive antigen test result varied in intensity from weak (1+, 2+) to strong (3+, 4+) according to the level of antigen in the serum.

**Statistical analysis.** All parameters were transferred to an IBM (Yorktown Heights, NY) PC/AT compatible computer for analysis using statistical analysis program package Instate Software for Science, version 2.3 (Graphpad Software, Inc., San Diego, CA). Non-parametric statistical methods were applied to evaluate the results. Concordance was assessed by Spearman’s rank correlation. P values are two-tailed and the significance of difference follow the following arbitrary scheme: *P* > 0.05 (not significant), *P* < 0.05 (significant), *P* < 0.01 (very significant), and *P* < 0.001 (extremely significant).

**RESULTS**

**Detection of Schistosoma antigen in serum and urine of infected pregnant women and controls.** The C5C4 mAb identified a polypeptide band with a molecular mass of 63-kD in serum samples collected from *S. mansoni*-infected pregnant women. No reactive bands were shown in the serum samples of uninfected pregnant women (Figure 1). This *Schistosoma* circulating antigen was also detected by the dot-ELISA in all urine and serum samples of 174 *S. mansoni*-infected pregnant women before delivery. A strong correlation was shown between the level of 63-kD antigen in serum and egg counts of infected women (*n* = 47; Spearman’s *ρ* = 0.72, *P* < 0.001). No false-positive results were found among 25 uninfected pregnant women used as controls.

**Schistosoma-specific antibody response.** Antibodies to *Schistosoma* SEA were detected by the indirect ELISA in all infected women (139 sera were positive for IgG and 35 sera were positive for both IgG and IgM). However, specific IgG antibodies to the 63-kD antigen were detected in 91% of the serum samples from 174 infected pregnant women. All 25 control sera from uninfected pregnant women were negative for both IgG and IgM antibodies to *Schistosoma*. No significant correlation was shown between the levels of specific IgG antibodies to the 63-kD antigen and the serum antigen levels.
Detection of Schistosoma antigen in infants after parturition. The 63-kD Schistosoma antigen was detected by the dot-ELISA and immunoblotting in the urine of all 25 infants born to S. mansoni-infected mothers seven days after parturition. However, this antigen was not detected in the urine of all 25 infants 28 days after parturition (Table 1). No false-positive results were found among five control mothers and their infants during these periods.

DISCUSSION

Vaccine development is being revolutionized by the ability of candidate antigens to induce cellular, and humoral immune responses against pathogenic viruses, parasites, and bacteria. However, newborns exposed to foreign antigens are at risk of developing tolerance rather than immunity due to the immaturity of their immune systems. Exposure to maternal antigens might predispose a child to develop an immunoregulated, asymptomatic clinical presentation of the infection, and may predispose them to respond with an altered, perhaps more immunoregulated, form of schistosomiasis. The probably immunogenic effects of such antigens as vaccines among infected mothers and the proper time to vaccinate newborns need to be studied.

Few reports have appeared in the literature suggesting that of infected women (n = 174; Spearman’s ρ = 0.09, P > 0.05). In addition, no significant correlation was shown between the levels of specific IgG antibodies to the 63-kD antigen and egg counts of the infected women (n = 47; Spearman’s ρ = 0.06, P > 0.05).

Detection of Schistosoma antigen and specific antibody in cord blood. Schistosoma antigen was detected by the dot-ELISA in 149 (86%) of 174 cord blood sera of S. mansoni-infected mothers. No false positive results were found in cord sera of 25 uninfected controls. The C5C4 mAb also identified by Western blotting a polypeptide band with a molecular mass of 63-kD in cord blood sera collected from 75 randomly selected S. mansoni-infected pregnant women. No reactive bands were shown in cord blood sera of 25 uninfected pregnant women (Figure 1). Specific IgG antibodies to SEA and the 63-kD antigen were detected in 81% and 80%, respectively, of cord blood sera from 174 infected pregnant women. These antibodies were not detected in cord blood sera of 25 uninfected controls.

Detection of Schistosoma antigen in breast milk and in urine of young children during the breast-feeding period. Western blotting identified the 63-kD Schistosoma antigen in urine samples of all 25 infants, as well as in breast milk and serum samples collected from their infected mothers during the breast-feeding period (18–24 months) (Figure 2). No false-positive results were found among five negative control mothers and their infants during this period. The dot-ELISA detected the 63-kD Schistosoma antigen in urine samples of all 25 infants born to S. mansoni-infected mothers, as well as in sera collected from their infected mothers during that period.

![Image of Western blotting](image-url)

**Figure 1.** Identification by Western blotting of the Schistosoma mansoni 63-kDa antigen in maternal blood serum and cord blood serum samples from infected mothers. A, Maternal blood serum. B, Cord blood serum. Lane 1, S. mansoni soluble egg antigen (SEA) (positive control); lanes 2–4, samples from three uninfected mothers; lanes 5–9, samples from five S. mansoni-infected mothers. Monoclonal antibody C5C4 reacted with a 63-kDa antigen in breast milk and serum samples from S. mansoni-infected mothers. No reaction was shown in samples from uninfected mothers. The molecular mass (Mr) protein markers (not shown but indicated by arrows) are phosphorylase B (Mr = 97.4 kDa), bovine serum albumin (Mr = 66.2 kDa), glutamate dehydrogenase (Mr = 55.0 kDa), ovalbumin (Mr = 42.7 kDa), aldolase (Mr = 40.0 kDa), carbonic anhydrase (Mr = 31.0 kDa), and soybean trypsin inhibitor (Mr = 21.5 kDa).

![Image of Western blotting](image-url)

**Figure 2.** Identification by Western blotting of the Schistosoma mansoni 63-kDa antigen in breast milk samples from S. mansoni-infected and uninfected mothers and in urine of their infants during the breast-feeding period and after parturition. Lane 1, S. mansoni soluble egg antigen (SEA) (positive control); lanes 2 and 3, breast milk samples from two uninfected mothers; lanes 4 and 5, breast milk samples from S. mansoni-infected mothers; lanes 6 and 7, urine samples from infants borne to the uninfected mothers; lanes 8 and 9: urine samples from two infants borne to infected mothers before parturition; lanes 10 and 11, urine samples from the two infants borne to the infected mothers 28 days after parturition. Monoclonal antibody C5C4 reacted with a 63-kDa antigen only in breast milk samples from S. mansoni-infected mothers and urine samples from their infants before parturition. The molecular mass (Mr) protein markers (not shown but indicated by arrows) are the same as those shown in Figure 1.
Detection of *Schistosoma* antigen by a dot-enzyme-linked immunosorbent assay in urine of 25 infants born to infected mothers after parturition

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*During the breast-feeding period (18–24 months) i.e., before parturition, the antigen was detected in urine of all infants. In addition, no false-positive results were found among five negative control infants during this period and post-parturition.

Maternal schistosome antigens are transferred through the placenta.15–17 Carlier and others,18 using polyclonal antibodies, detected a circulating *S. mansoni* antigen in sera from umbilical cords of newborns of *S. mansoni*-infected mothers. Hassan and others17 detected *Schistosoma* carbohydrate antigens by a capture ELISA in 66% of the urine samples from newborns of 45 *S. mansoni*-infected mothers. We have identified and characterized a 63-kD *Schistosoma* antigen in adult worms, eggs, and cercariae antigenic extracts of *S. mansoni* and *S. haematobium* using the C5C4 mAb.6 In the present work, we at first investigated the possibility of placental and oral delivery of a 63-kD maternal *Schistosoma* antigen. The placental delivery of intact 63-kD antigen was then evaluated. The 63-kD *Schistosoma* antigen was identified in umbilical cord serum and breast milk samples, as well as in serum and urine samples from *S. mansoni*-infected women. In addition, the natural immunogenicity of the 63-kD antigen was identified by the detection of the specific IgG antibodies to this antigen in 91% of the sera of infected pregnant women. However, no correlation was shown between the levels of specific IgG antibodies to the 63-kD antigen and the serum antigen levels of infected women. Although the relationship between the level of 63-kD antigen in sera of the infected women and egg counts was significant, the levels of specific IgG antibodies to the 63-kD antigen were not significantly correlated with the egg counts of 47 infected women. However, a large number of samples are needed to make any final conclusions.

The 63-kD antigen was detected in 86% of a large number of cord blood serum samples from infected women. Specific IgG antibodies to the 63-kD antigen were also detected in 80% of cord blood sera from infected pregnant women. The detection of the this *Schistosoma* antigen in sera from infected mothers and their cord blood sera demonstrates the transplacental transfer of the intact 63-kD antigen.

In the second part of the present study, 25 healthy infants were evaluated for the influence of their feeding pattern on the mother-to-child transmission of *S. mansoni* antigens. The 63-kD *Schistosoma* antigen was detected in the urine of the 25 newborns breast-fed by infected mothers that were followed two years after delivery. These data suggest that *S. mansoni* antigen can be transmitted in the colostrum/milk of infected mothers to their infants. Hassan and others17 hypothesized that a decrease of antigenemia (0% at six months of age) was due to the absence of a parasitic source of antigens in the infants and the elimination of schistosome antigens through the liver and kidneys within three months. In our study, the antigenemia persisted for 18–24 months, indicating the presence of another source of schistosome antigen delivery, i.e., breast milk. The 63-kD *Schistosoma* antigen was identified in the breast milk of the infected mothers. We confirmed the oral transfer of the intact *Schistosoma* antigen, in which the target antigen was not detected in the urine of some infants up to 21 days after parturition, then disappeared at 28 days.

Rueff-Juy and others18 strongly suggested that oral transfer of maternal immunoglobulins is more efficient at inducing and maintaining tolerance than placental transfer and that regulatory T cells with suppressor activity are involved in this process. The oral and placental transfer of maternal *Schistosoma* antigens might also maintain tolerance after future vaccination. Ichino and others19 examined the influence of a number of factors on the development of neonatal tolerance, including the nature, concentration, and mode of antigen presentation to the immune system, and the age of the host. They found that the induction of neonatal tolerance was critically dependent on the age of the recipient, the timing of vaccine administration, and the dose of vaccine, but was not restricted by the major histocompatibility complex. They also suggested that CD8+ T-cells were required for induction of tolerance.19 However, other mechanisms might account for the development of tolerance, and some of these involve cells that are not of the CD8+ phenotype.20

Thus, we have identified and confirmed the oral and placentation transfer of an intact *S. mansoni* antigen. Further studies to fully establish the mechanisms involved in the influence of placental versus oral delivery of *Schistosoma* antigens on immunoregulation are needed.

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