ENZYME-LINKED IMMUNOSORBENT ASSAY FOR IgA ANTIBODIES TO
TRYPANOSOMA CRUZI IN CONGENITAL INFECTION

M. CECILIA DI PENTIMA AND MORVEN S. EDWARDS
Department of Pediatrics, Baylor College of Medicine, Houston, Texas

Abstract. With the aim of achieving earlier diagnosis of congenital Trypanosoma cruzi infection, we assessed the usefulness of detecting specific IgA antibody by an ELISA. We evaluated 12 pregnant women chronically infected with T. cruzi, their newborn infants, and three additional neonates with parasitemia at birth. The IgA-specific antibody was detected by adapting the procedure for use of a commercial IgG ELISA, the Hemagen® Chagas’ Kit (Hemagen Diagnostics, Inc., Waltham, MA). Trypanosoma cruzi-specific IgA was detected in 10 (83%) of 12 mothers at delivery, in one of three parasitemic infants, and one of 12 newborns of the chronically infected women. Testing of 13 infants at six months of age revealed IgA in seven infants (54%), of whom four also had persistent T. cruzi-specific IgG. Detection of T. cruzi-specific IgA could provide a criterion for diagnosis of congenital infection in the absence of detectable parasitemia.

American trypanosomiasis, or Chagas’ disease, is caused by the protozoan parasite Trypanosoma cruzi. The disease is endemic in many parts of South and Central America. Serologic surveys indicate that 17–18 million individuals in Latin America are infected.1 As many as 30% of these will develop over time irreversible cardiac and hollow visceral lesions.

The diagnosis of congenital T. cruzi infection at birth relies on detection of the parasite in blood by microhematocrit, xenodiagnosis, or hemoculture.2–11 These tests are relatively insensitive. Several sensitive serologic techniques to detect antibodies against parasitic components are available, but the specificity of these methods is unsatisfactory.2 Since failure to isolate the parasite from the blood does not exclude congenital infection, serial serologic testing in infancy, measuring the level of T. cruzi-specific IgG, is performed.2 Persistent IgG antibody to T. cruzi at six months of age currently is an indication for therapy.2 We proposed that a screening method based upon detection of IgA antibodies, which are produced by the infant and not placentally transferred, might provide early and accurate diagnosis of Chagas’ disease in infancy. The method used was adapted from one currently in use for assessment of IgG-specific antibody to T. cruzi by an ELISA. We then evaluated the usefulness of detecting IgA-specific antibodies by ELISA for the diagnosis of congenital T. cruzi infection.

PATIENTS, MATERIALS, AND METHODS

Patients. From January 1, 1996 to January 30, 1997, pregnant women were assessed at the time of hospital admission for delivery at the maternity ward of the Maternal and Children’s Hospital in Salta, Argentina. The protocol was reviewed and approved by the Director of the hospital. Patients with positive IgG ELISA results for Chagas’ disease were enrolled in the study after informed consent for participation was obtained. Infants born to these women were evaluated clinically and serologically at birth and at 5–6 months of age. Clinical data were collected on a standardized form. Sera were collected at birth from 12 mother-baby pairs and from three neonates with parasitemia. Follow-up examination and serologic testing was performed for 13 of the 15 infants. A total of 12 cord blood sera, 20 sera from infants 1–6 months of age and 30 adult sera, all from healthy individuals living in Houston, Texas, were used as controls. The protocol for collection of the control sera was approved by the Institutional Review Board for Human Research at Baylor College of Medicine.

Determination of the presence of circulating antibodies to T. cruzi. The presence of IgA-specific antibodies to T. cruzi was determined by adaptation of a commercially available IgG ELISA, the Hemagen® Chagas’ Kit (Hemagen Diagnostics, Inc., Waltham, MA). In this assay, purified antigens extracted from cultured T. cruzi organisms were attached to the inner surface of each microwell. Detection of IgG-specific antibodies to T. cruzi was performed according to the manufacturer’s instructions. The IgA-specific antibodies were detected by substitution of peroxidase-conjugated sheep anti-human IgA (α chain) (The Binding Site, Ltd., Birmingham, United Kingdom) as the second antibody. Se- rial two-fold dilutions of each serum sample (1:4–1:128) to be tested for IgA were performed using phosphate-buffered saline (PBS) containing 5% newborn calf serum (Biowhittaker, Walkersville, MD) as the diluent. An aliquot (100 μl) of each serum sample was transferred to a microtiter well, incubated for 2 hr at room temperature, and washed with PBS. A 100-μl volume of anti-human IgA at a dilution of 1:6,400 in PBS was incubated for 2 hr at room temperature. The samples were then washed and 100 μl of 3,3′,5,5′-tetramethylbenzidine was dispensed and incubated for 10 min at room temperature. The reaction was stopped by adding 50 μl of 1 N sulfuric acid. The absorbance of each well was measured at 450 nm using an ELISA plate reader (MR 5000; Dynatech, Alexandria, VA). The mean optical density (OD) of negative control sera was calculated. Sera known to contain IgA-specific antibody to T. cruzi (provided by Dr. Greg Chiklis, Associate Director of Research and Development, Hemagen Diagnostics, Inc., Waltham, MA) were used as positive controls for standardization of the IgA-specific ELISA. Based upon evaluation of these IgA-rich sera, a dilution of 1:32 was chosen as optimal for detection of IgA antibody. Cord blood serum from a T. cruzi-negative infant and a pool of serum from five adults from the United States with no detectable IgA antibodies served as negative controls.

Results were expressed as the OD. The parameters set for defining the negative and positive cut-off values for the ELI-
SA were adapted from those provided with the Hemagen® Chagas’ kit for assessment of IgG antibody to T. cruzi. A negative value was defined as the OD of negative cord blood or adult sera plus 0.25. A specimen was considered positive for IgA when the OD exceeded the cut-off value plus 10%.

**Separation of IgG from IgA antibodies.** Human IgG antibodies from selected sera were separated using Quick-Sep® System II IgM/IgG Isolation (Isolab, Inc., Akron, OH). This ion-exchange–based method separates serum into an IgG fraction containing IgG1, IgG2, and IgG3, and an IgM fraction that contains IgM, IgG4, and other serum proteins, including IgA. Samples were processed according to the manufacturer’s instructions. Briefly, an aliquot of 100 μl of six representative infant or adult sera with detectable IgA was diluted with 1 ml of 0.15 M IgG wash buffer, and introduced into the chromatographic column provided. The IgG was eluted with 9 ml of 0.15 M IgG wash buffer. The IgA and IgM then were eluted with 2 ml of 0.15 M IgM elution buffer. The samples were concentrated to the starting volume in a Minicon B15 macrosolute concentrator (Amicon Inc., Beverly, MA), dialyzed against saline to remove sodium azide, and tested semiquantitatively by serial two-fold dilution for the presence of IgA- and IgG-specific antibodies to T. cruzi.

**Statistical analysis.** Statistical significance was determined using the unpaired t-test. P values < 0.05 were considered significant. Analysis was performed using Epi-Info Version 6.0 (USDI, Inc., Stone Mountain, GA) and SPSS Version 7.0 (SPSS Inc., Chicago, IL) software packages.

**RESULTS**

**Patients.** Pertinent epidemiologic and clinical data for the 15 newborn infants evaluated are summarized in Table 1. Each of the mothers of these infants had IgG-specific antibodies to T. cruzi detected by ELISA during pregnancy. Three neonates had parasitemia confirmed by microhematocrit and were symptomatic at birth. Two had hepatomegaly, and one was severely ill with pneumonia and meningitis due to T. cruzi. Among the 12 infants born to chronically infected women, nine were asymptomatic. At six months of age, each of the 12 infants was asymptomatic. Of the three infants with parasitemia at birth, one was available for assessment at six months of age. Physical examination revealed persistent hepatosplenomegaly.

**IgG-specific antibodies to T. cruzi.** Each pregnant woman and her newborn had IgG-specific antibodies to T. cruzi detectable by ELISA at delivery or in cord serum. At 5–6 months of age, six (46%) of the 13 infants had persistent IgG-specific antibodies (Figure 1). Controls had undetectable T. cruzi IgG-specific antibodies.

**IgA-specific antibodies to T. cruzi.** IgA-specific antibody to T. cruzi was detected in one (33.3%) of three infants with parasitemia at birth, and one (8%) of 12 newborns exposed to chronically infected mothers (Figure 1). At 5–6 months of age, follow-up testing results in 13 infants was positive for IgA in seven (54%), of whom four (57%) had persistent IgG. Of the infants who were seronegative for IgA at six months, four also were IgG negative (Figure 1). *Trypanosoma cruzi* IgA-specific antibodies were detected in the sera of 10 (83%) of 12 mothers tested at delivery.

Each serum from 12 control neonates was negative for IgA-specific antibodies. The mean OD for these newborn sera was 0.005 (Figure 2). The mean OD for sera from 15 infants born to chronically infected mothers (12 infants) and those with parasitemia (three infants) was significantly higher (0.129; P = 0.004). One of 20 sera from 5–6-month-old control infants was positive. At 5–6 months of age, the mean OD for the 13 exposed infants for whom follow-up sera were available (0.419) was significantly higher than that for 20 unexposed control infants (mean = 0.162; P = 0.001) (Figure 2).

Sera from 30 healthy adults were tested for IgA-specific antibody to T. cruzi. The mean OD for these sera was 0.292 (range = 0.058–0.517), while that in delivery sera from the 12 pregnant women with chronic Chagas’ disease (mean = 0.473, range = 0.267–0.824) was significantly higher (P = 0.005).

**Removal of IgG antibodies.** Sera from three infants and three chronically infected women, each with detectable IgA, were processed to separate the IgA from the IgG. Samples were dialyzed against saline after the separation procedure to remove residual sodium azide that might interfere with the enzymatic reaction. Repeat testing for IgA-specific antibody to T. cruzi revealed a maximal change in titer of one dilution (Table 2). This provided additional confirmatory evidence that the modified ELISA for detecting IgA antibody was specific.

**DISCUSSION**

Congenital infection due to *T. cruzi* is acquired through placental passage of maternal parasitemia. The associated
risk for the fetus is related to the degree of maternal parasitemia. In Latin America, the estimated prevalence of *T. cruzi* antibodies in sera from pregnant women ranges from 2% to 51%. The incidence of congenital infection ranges from 3% to 5%. The most seriously ill infants have hemorrhagic symptoms that can result in a fatal outcome. The majority of infants with congenital Chagas’ disease are asymptomatic during the first few months of life, but they may develop hepatosplenomegaly, dysphagia, or seizures in subsequent years or classic chronic Chagas’ disease. The contribution to the total Chagas’ disease burden of untreated congenital infection is not fully defined, but these children are believed to contribute substantially to the pool of chronic infection.

Since IgA is produced early in fetal life and it does not cross the placenta, detection of IgA-specific antibody has been extensively investigated for the early diagnosis of congenital infections. For example, an IgA antibody response to *Toxoplasma gondii* antigens is detected more often than IgM antibody in congenital infection and it persists longer. However, few studies have been published in English with regard to IgA-specific antibodies to *T. cruzi* and their role in the diagnosis of congenital infection. The most comprehensive is the recent report from Chile of Lorca and others who evaluated the IgA and IgM response in 24 chronically infected mothers and their infants. By conventional immunofluorescent antibody assay and ELISA, *T. cruzi*-specific IgA was detected in three of 12 infected newborn infants at birth. However, two had borderline results. The use of recombinant antigens improved the sensitivity of the test, so that five infants had detectable IgA-specific antibodies at birth, and three others became positive within two months.

Our results confirm that the detection of IgA-specific antibodies to *T. cruzi* at birth by ELISA, although highly specific, has low sensitivity for the diagnosis of congenital Chagas’ disease at birth. The IgA-specific antibodies to *T. cruzi* were detected in two (13%) of 15 infants born to chronically infected pregnant women. Acquisition of infection late during pregnancy or intrapartum, rather than transplacental passage, might be an explanation for the lack of sensitivity of the test in newborn infants. Additional evaluation would

### Table 2

<table>
<thead>
<tr>
<th>Serum source</th>
<th>Reciprocal dilution positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant</td>
<td>Whole serum</td>
</tr>
<tr>
<td>Infant</td>
<td>8</td>
</tr>
<tr>
<td>Infant</td>
<td>64</td>
</tr>
<tr>
<td>Infant</td>
<td>32</td>
</tr>
<tr>
<td>Adult</td>
<td>16</td>
</tr>
<tr>
<td>Adult</td>
<td>32</td>
</tr>
<tr>
<td>Adult</td>
<td>32</td>
</tr>
</tbody>
</table>
then be required in the months following birth to exclude the diagnosis of congenital infection. Although the sensitivity of the assay described here improves by six months of age, it still is not optimal. Further testing at 12 months of age might be required to accurately distinguish between infants with passively acquired IgG antibodies to *T. cruzi* and those with true infection as indicated by IgA-specific antibodies.

The early diagnosis of congenital Chagas’ disease is crucial to instituting appropriate therapy during the acute phase of the disease. We concur with others that detection of IgA-specific antibodies to *T. cruzi* in infants beyond the neonatal period may be a useful method by which to identify infants at risk for congenital infection, in whom persistence of IgG does not allow unequivocal differentiation between infection and maternally transferred antibodies.

Acknowledgments: We acknowledge the helpful comments of Dr. Carol J. Baker in reviewing the manuscript and Robin D. Schroeder for assistance in its preparation. We are also grateful to Dr. Mario Martin and Dr. Marcela Belmonte for assisting with field collection of samples. The ELISA plates required to conduct these studies were subsidized in part by Hemagen Diagnostics, Inc. (Waltham, MA).

Authors’ addresses: M. Cecilia Di Pentima, Sarmiento 229, Junin, Buenos Aires, Argentina. Morven S. Edwards, Department of Pediatrics, Section of Infectious Diseases, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030.

Reprint requests: Morven S. Edwards, Department of Pediatrics, Section of Infectious Diseases, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030.

REFERENCES