Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 2846–2850, April 1990
Medical Sciences

Fetal IgG specificities against *Trypanosoma cruzi* antigens in infected newborns

(Chagas disease/congenital infections/IgM/recombinant DNA)

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Communicated by Cesar Milstein, January 16, 1990

ABSTRACT **A panel of *Trypanosoma cruzi* antigens produced by recombinant DNA techniques was used to analyze the IgM and IgG specificities present in sera from 22 mothers with chronic Chagas disease and their newborn infants. Ten of the newborns were congenitally infected and the other 12 children were healthy. While in most cases IgG specificities in the newborns mirrored those of their mothers, congenitally infected newborns had, in addition, IgG specificities that were undetectable in their mothers. The new IgG specificities observed most frequently were against a shed acute-phase antigen (SAPA), and less frequently, against other nine different parasite antigens. Thus, SAPA is able to identify new fetal IgGs because antibodies against this antigen are generated during the acute phase of the infection and not in their chronically infected mothers. Sera from congenital cases also had IgMs against several parasite antigens, but again, SAPA was the most frequently detected. Neither IgMs nor new IgG specificities were detected in healthy children born to mothers with Chagas disease. We conclude that individual antigens can be used to detect new IgG specificities present in the cord blood from infected newborns. Furthermore, detection of IgMs and new fetal IgGs with recombinant antigens may be used to sort out congenitally infected infants from uninfected ones, a method that might be applied to other infectious diseases.**

IgM, IgA, and IgE antibodies do not cross the placenta (see refs. 1 and 2 for recent reviews) and their presence in the cord blood is the consequence of their production by the fetus. Thus, detection of these antibody classes is suggestive of active infection and has been of great use in the diagnosis of congenital infection in cases of filariasis (IgE detection) (3), syphilis (IgM detection) (4, 5), and toxoplasmosis (IgM and IgA detection) (6–8) among other infections in newborn humans (see ref. 2 for a review). The situation with antibodies of the IgG class is different. All four IgG subclasses cross the placenta and are detectable in the fetus from weeks 22–24 of gestation due to passive and active transport from the mother's blood (see refs. 1 and 2 for recent reviews). In accordance, the IgG Gm phenotype of the newborn is always similar to that of its mother (2, 9). However, early reports showed that *in vitro* cultured cells from spleen fetuses are capable of IgG synthesis from midgestation (10). Furthermore, trace amounts of IgGs carrying Gm markers not present in the corresponding maternal serum were observed in cord sera (9, 11). This small fraction of IgG molecules is supposed to represent the child's own Gm phenotype and thus they are probably synthesized by the fetus *in utero* (see refs. 12 and 13 for reviews). The contribution of the fraction produced by the fetus to the IgG serum level is negligible and thus most IgG detected in cord blood is of maternal origin (1, 2, 14, 15). Due to the above considerations, detection of specific fetal IgGs in the cord blood of newborns that are congenitally infected may not be easy. Attempts to detect in the cord blood IgG specificities against pathogens that are not present in the corresponding maternal blood were made in congenital infections of toxoplasmosis, using immunoelectrophoresis (16) and Western blots of total parasite proteins (7), and also in congenital cases of syphilis, using Western blot analysis (5). However, confirmation of specific and unique antigen–antibody reactions in infected newborns may be complicated when these approaches are used (7).

Chagas disease is a severe endemic problem, caused by the parasitic protozoan *Trypanosoma cruzi*, that affects several million people in Central and South America. Diagnosis is based on the detection of antibodies against *T. cruzi* through the use of killed parasites or parasite fractions (17). This complex mixture of antigens does not allow detection of variations in antibody specificities in acute, chronic, and congenital infections. Our group (18, 19) has cloned and sequenced several DNA fragments encoding different parasite antigens. One of the recombinant antigens, shed acute-phase antigen (SAPA), detected antibodies in 93% of sera from patients at the acute phase of the infection but in only 10% of patients at the chronic phase of the disease (20). On the other hand, recombinant antigens 1, 2, and 30 detected antibodies mainly in sera from chronic patients. Still a third group of antigens, 13 and 36, detected both acute and chronic phases of the disease. Since SAPA is a specific marker of acquired acute infection in adults, it should help in the identification of acute congenital cases. Furthermore, mothers are more frequently at the chronic phase and thus not producing antibodies against SAPA. Consequently, antibodies against this antigen in infected newborns might indicate their active production by the fetus. In this study, 11 *T. cruzi* antigens produced by recombinant DNA techniques were used to analyze the antibody specificities in sera from congenitally infected newborns and their mothers. We show that it is possible to use individual antigens to detect the specific fetal IgGs present in the cord blood of congenitally infected newborns.

MATERIALS AND METHODS

Patients and Sera. Forty-four blood samples were collected. Twenty samples were from umbilical cord at birth, 8 from congenital Chagas disease and 12 without infection. Two other samples from congenital cases were obtained from infants 2 days (case 21) and 2 months (case 22) after birth. Sera from the 22 corresponding mothers, all of whom had chronic Chagas disease, were collected at the same time as their children. Two serological methods were used to examine the serial dilution of serum samples. The ELISA technique of Voller (21) was used with soluble extracts from

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Abbreviation: SAPA, shed acute-phase antigen.

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cultured epimastigotes of *T. cruzi* as antigen and rabbit anti-human IgM or IgG conjugated with peroxidase (Sigma) as secondary antibody. The indirect immunofluorescence technique was applied as described by Camargo (22), with fixed cultured epimastigotes as antigen and anti-human IgM or IgG conjugated with fluorescein isothiocyanate. Two xenodiagnoses were applied to all children at birth (or at the time of blood sampling, for infants 21 and 22) and were then examined at 30, 60, and 90 days as described by Schenone *et al.* (23). Xenodiagnosis consists in the application of laboratory-reared triatomines on the patient and the examination of the triatomines' feces (24). All the congenitally infected infants were positive by xenodiagnosis at the indicated days after birth, whereas all the uninfected infants were negative (Table 1). Clinical examination was also performed at birth (or at the time of blood sampling, for infants 21 and 22). Eight congenital cases presented clinical manifestations, five of them hepatosplenomegaly (cases 14, 17, 19, 20, and 22), three others had encephalitis signs (cases 14, 19, and 21), and two others had anemia (cases 16 and 22). The uninfected infants were healthy at birth.

Production of *T. cruzi* Antigens from Recombinant DNAs and Detection of IgM and IgG. Eleven cloned *T. cruzi* antigens were used. Nine of them were previously characterized and from eight of them, partial DNA sequence was obtained (18–20). The inserts from two new recombinant antigens (nos. 19 and 49) have not been characterized. To test sera against the 11 recombinant *T. cruzi* antigens, nitrocellulose filters containing isopropyl β-D-thiogalactopyranoside were placed over plaques of λgt11 recombinant phages. The phages were grown on a lawn of *Escherichia coli* Y1090. λgt11 without insert was used as control. Filters were then processed with the indicated sera followed by IgG made in rabbits against human IgM (μ-chain specific; Sigma) or

Table 1. Description of uninfected and congenitally infected infants born to mothers with Chagas disease

Newborn status	Case	Serum		IgG titer		IgM titer		Xenodiagnosis‡	Symptoms
		Number	Origin*	ELISA	IIF†	ELISA	IIF†		
Uninfected	1	87/1887	UC	ND	80	–	–	–	–
		87/1889	M	ND	160	–	–		
	2	87/1413	UC	ND	80	–	–	–	–
		87/1414	M	ND	160	–	–		
	3	87/1928	UC	ND	80	–	–	–	–
		87/1890	M	ND	160	–	–		
	4	88/1986	UC	ND	640	–	–	–	–
		88/1987	M	ND	160	–	–		
	5	88/2001	UC	ND	160	–	–	–	–
		88/1997	M	ND	320	–	–		
	6	87/1332	UC	ND	160	–	–	–	–
		87/1327	M	ND	80	–	–		
	7	87/1417	UC	ND	80	–	–	–	–
		87/1420	M	ND	160	–	–		
	8	87/1701	UC	ND	80	–	–	–	–
		87/1526	M	ND	320	–	–		
	9	88/2000	UC	ND	160	–	–	–	–
		88/2005	M	ND	320	–	–		
	10	88/1883	UC	ND	320	–	–	–	–
		88/1881	M	ND	320	–	–		
	11	88/1840	UC	ND	320	–	–	–	–
		88/1844	M	ND	640	–	–		
	12	88/1410	UC	ND	160	–	–	–	–
		88/1325	M	ND	320	–	–		
Infected	13	87/1710	UC	160	160	80	–	+ (60)	+
		87/1711	M	1,280	160	160	–		
	14	87/1333	UC	640	1280	160	–	+ (30)	+
		87/1329	M	1,280	320	2000	10		
	15	86/196	UC	1,280	160	40	–	+ (30)	+
		86/195	M	1,280	1280	320	10		
	16	88/2052	UC	640	320	2000	–	+ (30)	+
		88/2235	M	16,000	160	640	–		
	17	88/280	UC	80	320	–	–	+ (90)	+
		88/279	M	160	640	80	–		
	18	88/169	UC	20	20	640	–	+ (60)	–
		88/1718	M	1,280	640	1280	–		
	19	88/188	UC	160	160	–	–	+ (30)	+
		88/147	M	32,000	160	2000	–		
	20	88/2215	UC	1,280	20	–	–	+ (90)	–
		88/1622	M	1,280	160	80	–		
	21	83/1015	I	4,000	80	–	–	+ (30)	+
		83/1016	M	4,000	320	640	20		
	22	2E/43	I	640	10	640	–	+ (30)	+
		2E/44	M	2,000	1280	320	40		

ND, not done.
*UC, umbilical cord; M, mother; I, infant [2 days (case 21) or 2 months (case 22) after birth].
†IIF, indirect immunofluorescence.
‡Numbers in parentheses indicate the time (days) required for a positive diagnosis (see ref. 24).

Table 2. IgM and IgG specificities in sera from infected and uninfected newborns and their mothers

Newborn status	Antibody class	Serum source	No. of sera tested	No. of positive sera	No. of sera positive with each antigen										
					1	2	SAPA	10	13	26	30	36	54	19	49
Infected	IgM	Newborns	10	8	2	1	7	0	5	0	0	0	0	1	0
		Mothers	10	2	0	1	1	0	0	0	0	0	0	0	0
	IgG	Newborns	10	10	8	5	7	2	5	2	6	6	0	5	0
		Mothers	10	10	9	7	0	1	3	2	6	3	1	3	0
Uninfected	IgM	Newborns	12	0	0	0	0	0	0	0	0	0	0	0	0
		Mothers	12	4	0	1	0	0	3	0	0	0	0	0	0
	IgG	Newborns	12	12	10	11	1	2	10	1	7	7	0	6	0
		Mothers	12	12	10	11	1	2	11	4	7	8	2	7	2

human IgG (γ-chain specific; Sigma) labeled with ^{125}I and Iodo-Gen (Pierce). Control experiments showed that under the conditions used, rabbit antibodies against human IgM and IgG detected the corresponding purified human antibodies (data not shown). Filters were processed as previously described in detail (18), except for the use of 3% nonfat dried milk and 2% glycine as blocking agents instead of 3% bovine serum albumin. All sera from human infections were used in 1:100 dilution unless otherwise stated. Recombinant phage plaques showing reactions stronger than the background observed with λgt11 were recorded as positive. Serum samples were sent coded and were uncoded after processing.

RESULTS AND DISCUSSION

Sera from 10 congenitally infected and 12 uninfected newborns were analyzed together with sera from their mothers (Table 1). Sera were collected from infants at birth from the umbilical cords except for cases 21 and 22, in which sera were obtained 2 days and 2 months after birth, respectively. All 12 uninfected infants had IgG antibodies to *T. cruzi* antigens but were negative for IgM and by xenodiagnosis (24). All 10 congenitally infected infants had detectable IgG levels, 6 out of 10 had IgM detectable by ELISA but not by indirect immunofluorescence, and all were positive by xenodiagnosis. Since the mothers studied were at a chronic stage of the infection, it is surprising that sera from all the mothers whose offspring were infected had IgM antibodies in ELISA tests, while none of the mothers delivering healthy children had IgM responses (Table 1). We do not have an explanation for this finding but it might be the result of a nonspecific stimulation of the maternal immune system as a consequence of the fetal infection. It is noteworthy that these results are just the opposite of those obtained when recombinant *T. cruzi* antigens were used to detect IgM (Table 2). In the latter case, only 2 out of 10 mothers were positive with one antigen each (Table 2), outlining the importance of individual antigens for antibody detection (see below).

Sera were coded before they were processed with the filters containing recombinant *T. cruzi* proteins. Nine previously described recombinant *T. cruzi* antigens (18–20) together with two new ones (antigens 19 and 49) were used to detect IgM and IgG specificities in sera from infected newborns and their mothers. IgM and IgG specificities in sera from four cases are shown in Fig. 1*A*, and all the results are summarized in Tables 2 and 3. Two of the mothers had detectable IgM, and all had IgG with various specificities, which detected mainly antigens 1, 2, 13, 30, and 36 (Table 2). The latter is a typical response expected from chronic cases of the disease (19, 20). Analysis of the sera from the 10 corresponding infected newborns showed that 8 of them had IgM specificities against several *T. cruzi* proteins (Table 2),

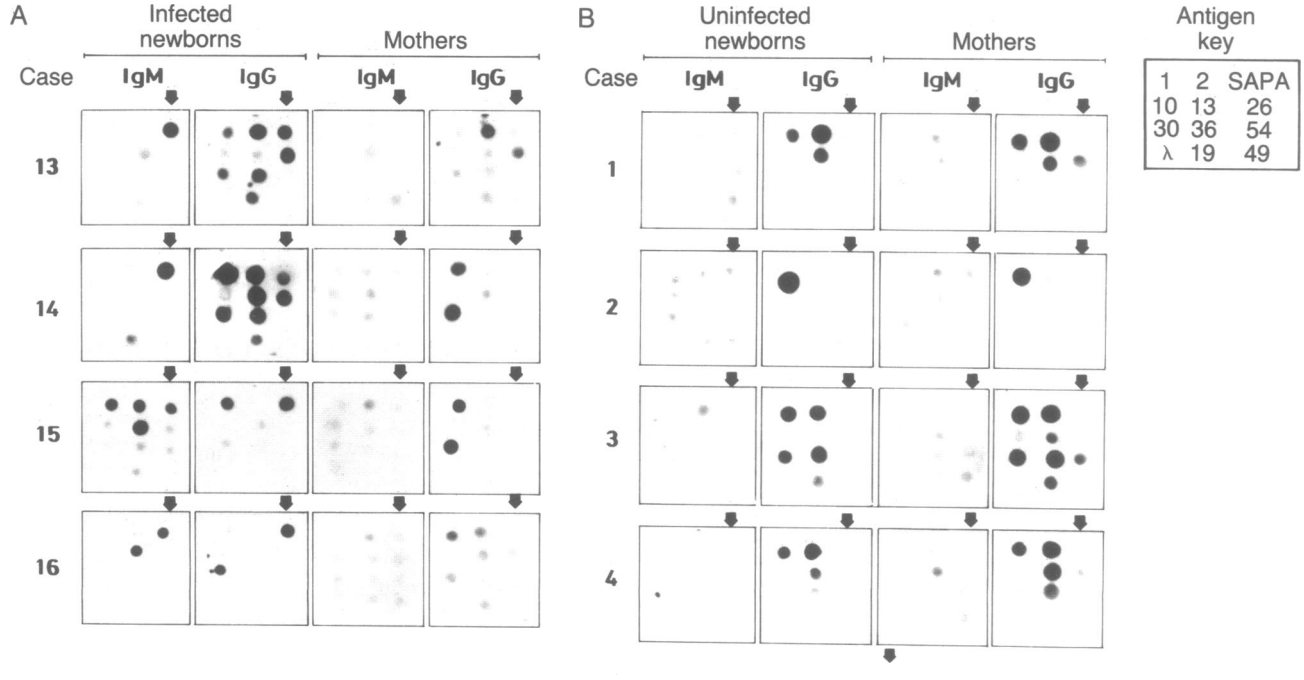

FIG. 1. IgM and IgG specificities in sera from infected (*A*) and uninfected (*B*) newborns and their mothers. Eleven recombinant λgt11 clones encoding the indicated *T. cruzi* antigens were distributed in Petri dishes as shown at right. The reactivity of the parasite antigens with sera (1:100 dilution) from four pairs of maternal and umbilical cord sera is shown. Arrow points to SAPA; λ represents λgt11 control.

Table 3. IgG specificities in sera from infected and uninfected newborns that were not detected in sera from the corresponding mothers

Serum source	No. of sera tested	No. of positive sera*	No. of sera positive with each antigen*										
			1	2	SAPA	10	13	26	30	36	54	19	49
Infected newborns	10	9	1	2	7	1	2	1	2	4	0	3	0
Uninfected newborns	12	0	0	0	0	0	0	0	0	0	0	0	0

*IgG specificities detected in newborns and not in their mothers.

thus confirming active infection detectable at birth. Antibodies most frequently observed were against SAPA (7 cases) and antigen 13 (5 cases). Sera from congenital cases also had IgGs against several parasite antigens. Although some of these IgGs were against the same antigens as the ones detected by the corresponding maternal antibodies (Fig. 1*A* and Table 2), we found in addition IgG specificities in 9 out of 10 infected newborns that were not detected in the chronically infected mothers (Fig. 1*A* and Table 3). The new IgG specificity most frequently observed in the infected infants was against SAPA (7 out of 10 cases). Other new specificities were against antigens 36 (4 cases), 19 (3 cases), 2, 13, and 30 (2 cases each), and 1, 10, and 26 (1 case each).

The detection of new IgG specificities in newborns may be the consequence of a higher concentration of specific antibodies in blood from the umbilical cord than in the blood from the corresponding mother. For example, levels of IgG4 against varicella–zoster antigens were found to be 1.64 times higher in the cord blood than in the maternal blood (25). To test this possibility, the reactivity of serum dilutions from 2 congenitally infected newborns was analyzed (Fig. 2). No attempts were made to concentrate sera from the mothers, since we know from previous work that dilutions of 1:100 give clearer results over the background (19, 20). Sera from the two infected newborns diluted 1:100, 1:300, 1:600, and 1:1200 were clearly positive with SAPA. Reactivity against antigen 19 was also observed up to a dilution of 1:600 in case 13 (Fig. 2). On the other hand, 1:100 dilutions of sera from the corresponding mothers were negative with these antigens. Consequently, these results strongly suggest that new IgG specificities are indeed present in the sera from the newborns. The other result that supports this finding was obtained from studies on the antibody specificities in sera from 12 uninfected children born to mothers having Chagas disease. The reactivities obtained with sera from 4 cases are shown in Fig. 1*B*, and all the results are summarized in Tables 2 and 3. None of the sera from the 12 uninfected newborns had IgM against any of the antigens tested (Table 2). This result was expected and confirmed the absence of infection as previously diagnosed by conventional serology and xenodiagnosis (Table 1). All 12 uninfected newborns had IgG specificities similar to those present in their mothers (Fig. 1*B* and Tables 2 and 3). Unlike the infected newborns, none of the healthy children had IgG specificities not detectable in their mothers. In some cases, IgG antibodies against recombinant antigens were detected in the mother's serum but not in the corresponding offspring's serum (Table 2). This result was observed in both groups, that is, in the mothers delivering infected children as well as in those delivering healthy ones. Although several possible explanations may be envisaged, such as differential placental transfer of IgG subclasses, together with a strong isotype specificity, or variations in the period of infection of the fetus, further work should be done to explain this observation.

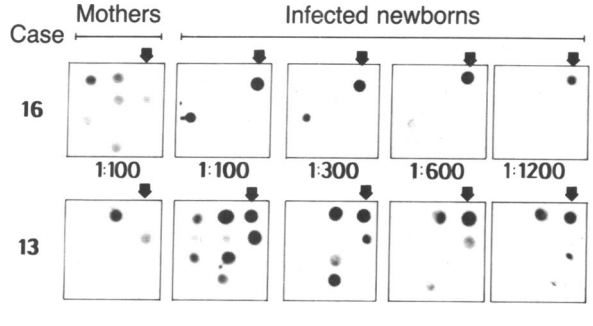

FIG. 2. IgG specificities detected in sera (1:100 dilution) from mothers and in sera (various dilutions) from their infected newborns. See Fig. 1.

These results show that it is possible to detect IgG specificities unique to sera from infected newborns when purified antigens are used. Further, these findings strongly suggest that the fetus is able to produce IgG antibodies during infections *in utero*. The observations made in congenital newborns and their mothers are in agreement with previous work from our laboratory showing that antibodies against SAPA were present in acute infections in adults, while they appeared in few of the patients at the chronic phase of Chagas disease (20). Interestingly, previous analysis of acquired infections in adults was done in patients infected in Brazil (20), while the present study of congenital infections was carried out in patients from Chile. SAPA is a main antigen during acute infections in these two groups of patients. Thus, these results are not dependent on the *T. cruzi* isolates present in the two countries.

Congenital cases of Chagas disease are identified in 50–60% of the cases through detection of IgM by ELISA (Table 1). Alternatively, xenodiagnosis may be used, but results are obtained 1–3 months later (24). The trypanocidal drugs available are effective during the early stages of the infection (26); hence, a method for early detection of infected newborns is essential for treatment. The use of recombinant antigens in the detection of specific IgM and new IgG specificities allowed us to identify 9 of 10 congenital cases. Therefore, this method is more powerful than ELISAs done with conventional antigens, where only 6 out of 10 congenital cases were identified (Table 1). Furthermore, when complex mixtures of antigens are used for IgM detection in congenitally infected infants, the presence of rheumatoid factors may obscure the results (5, 27). In our study with purified antigens this is not the case, since fetal IgMs against maternal IgGs were not detectable in healthy newborns who also acquired IgGs passively from their infected mothers. Thus, detection of specific IgMs and new IgG specificities provides an approach for early diagnosis that might be applied to other infectious diseases.

We thank O. Burrone and E. Jazin for critical reading of the manuscript and S. Leguizamon and O. Campetella for helpful suggestions. This work was supported by grants from the Special Programme for Research and Training in Tropical Diseases, World Health Organization and Rockefeller Foundation; Departamento Técnico de Investigación de la Universidad de Chile; Fondo de Ciencia y Tecnica de Chile; and the Swedish Agency for Research Cooperation with Developing Countries. M.B.R. is a research fellow and A.C.C.F. is a researcher from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

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