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Evaluation of Immunoglobulin M Western Blot Analysis in the Diagnosis of Congenital Syphilis

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Western immunoblots of solubilized *Treponema pallidum* antigens were reacted with sera and cerebrospinal fluid (CSF) and developed with enzyme-conjugated antibodies to immunoglobulin M (IgM). A blot was considered positive if reactions included bands at the 47-, 17-, and 15.5-kilodalton positions along with a variable pattern at other low-molecular-weight positions. Sera from 23 of 25 symptomatic infants diagnosed with congenital syphilis yielded positive reactions. Of 80 asymptomatic infants considered at risk for developing symptomatic infection, 16 exhibited IgM patterns consistent with those seen in congenital syphilis, although 5 of these 16 gave reactions that were equivocal. To exclude false-positive reactions due to IgM rheumatoid factor, sera were fractionated and the IgM fractions were retested. Only the five initially equivocal sera gave nonreactive blots with the IgM fractions, whereas all others gave more prominent reactions that were qualitatively similar to those seen in serum samples. Sera from 18 normal infants failed to show any IgM reactivity to *T. pallidum* antigens on Western blots. The IgM Western blot was both more sensitive and more specific than the fluorescent treponemal antibody-absorbed (IgM) test using fractionated serum. Of the 17 CSF samples from infants with symptomatic congenital syphilis, 14 showed IgM reactivity in Western blots, whereas only 12 had a reactive CSF in the Venereal Disease Research Laboratory test. Our results indicate that this technique can be used to identify both symptomatic and asymptomatic infection in infants with *T. pallidum*, in some cases before standard serologic studies can confirm the diagnosis.

Despite the fact that congenital syphilis is largely a preventable disease, it continues to be an important public health problem both in developing and developed countries (13, 16, 17, 26, 30). In the past, its diagnosis has been based on a combination of clinical, serological, and epidemiological findings and often was a retrospective diagnosis in those infants asymptomatic at the time of delivery. Transfer of maternal immunoglobulin G (IgG) antibodies across the placenta makes serologic diagnosis of infected infants unreliable, especially in those neonates who are asymptomatic at birth or in whom symptoms may be related to prematurity or other medical problems. Most hospital laboratories utilize both nontreponemal serologic tests for syphilis (STS), such as the Venereal Disease Research Laboratory (VDRL) test or the Rapid Plasma Reagin test, and confirmatory specific treponemal serology, such as the fluorescent treponemal antibody-absorbed (FTA-ABS) test or the microhemagglutination assay for *Treponema pallidum* antibody (33). These tests are of little practical value in the infant, since they measure primarily IgG antibodies and therefore are more reflective of maternal than neonatal infection.

Development of an FTA-ABS (IgM) test over 20 years ago (27) for sera of infants was initially heralded as a means of circumventing problems associated with passively acquired maternal IgG. Critical evaluation of the assay, however, revealed a problematic lack of standardization, in addition to unacceptably high false-positive and false-negative rates (14). Despite efforts to improve the assay, such as IgM fractionation of sera prior to testing (18), tests such as the FTA-ABS IgM and the FTA-ABS 19S IgM are still considered experimental rather than standard laboratory tests (6). Thus, their place in the clinical practice of pediatrics remains questionable.

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In response to a dramatic 32% rise in infectious (adult) syphilis reported in 1987 (5) and an increasing number of reported cases of congenital syphilis beginning in 1984 (7), the Centers for Disease Control, Atlanta, Ga., called for improved research efforts in the prevention and pathophysiology of syphilis. Some investigators have studied IgM responses to nontreponemal (23) and treponemal (9, 19) antigens by enzyme-linked immunosorbant assay, while others, including our laboratory, have adapted protein immunoblot (Western blot) techniques to identify *T. pallidum* antigens recognized by IgG and IgM (1, 3, 8, 25). The present study was undertaken in an attempt to simplify the Western blot assay and to expand the scope of our original study (8). Because the diagnosis of congenital syphilis is less problematic in infants with symptoms of infection at birth, the antibody responses of these infants were compared with those of asymptomatic infants at risk for congenital syphilis and those of infants not thought to be at risk. Efforts to avoid interference from maternal IgG and rheumatoid factor (RF) were made. Western blot results were compared with those obtained using a modified FTA-ABS (IgM) assay in an attempt to assess the sensitivity and specificity of the test in relation to previously described techniques.

MATERIALS AND METHODS

Patients. Serum and cerebrospinal fluid (CSF) samples were collected from the serology laboratories of local hospitals when newborns born to mothers with a reactive STS were evaluated or when older infants were admitted for suspected congenital syphilis. Control sera and CSF were obtained from newborns whose mothers had a nonreactive STS and no history of syphilis but had samples collected for other reasons (normal newborns). Samples were collected from September 1985 through December 1988, divided into aliquots, and stored at −70°C until used. Repeated freeze-

TABLE 1. Clinical and serological data for symptomatic infants

Patient	Gestation age (wk)	Age (days) at diagnosis	Reciprocal titer[a]			Symptoms
			RPR (infant serum)	VDRL (infant CSF)	RPR (maternal serum)	
1	39	1	256	1	256	Hepatosplenomegaly, osteochondritis, pneumonia
2	40	52	128	0	128	Hepatosplenomegaly, rash, rhinitis, hemolytic anemia
3	36	1	128	2	256	Hepatosplenomegaly, osteochondritis, rash
4	40	1	16	1	64	None
5	25	1	32	4	64	Hepatosplenomegaly, jaundice
6	42	54	8	0	64	Rash, rhinitis, osteochondritis
7	34	1	8	0	32	Small for gestational age, thrombocytopenia, hepatitis, hematuria
8	34	1	128	2	32	Hepatosplenomegaly, jaundice, rash, osteochondritis (?)
9	33	1	32	0	64	Hepatosplenomegaly with hepatitis, thrombocytopenia, leukocytosis
10	26	5	128	8	64	Hepatosplenomegaly, thrombocytopenia, osteochondritis
11	31	1	32	2	64	Jaundice, rash
12	32	1	256	4	32	Hepatosplenomegaly, jaundice, thrombocytopenia, leukocytosis, osteochondritis
13	26	5	4	0	64	Thrombocytopenia
14	40	2	128	2	128	Hepatosplenomegaly, jaundice, osteochondritis, rash, leukocytosis
15	40	1	64	0	128	Hepatosplenomegaly, jaundice, hepatitis
16	28	6	16	0	64	Thrombocytopenia, osteochondritis
17	40	3	1	32	4	None
18	40	66	128	0	128	Hepatosplenomegaly, jaundice, osteochondritis, condylomata lata, irritability
19	34	2	64	1	64	Hepatosplenomegaly, jaundice, thrombocytopenia, leukocytosis osteochondritis, right upper lobe pneumonia
20	36	2	128	8	512	Jaundice, pleocytosis, irritability
21	40	1	4	0	8	Single ulcer (sole of foot)
22	35	1	64	1	128	Hepatosplenomegaly, desquamating rash
23	37	1	64	4	64	Hydrops, ascites, hepatosplenomegaly, jaundice, hemolytic anemia, respiratory distress
24	36	1	1,024	64	64	Hepatosplenomegaly, jaundice, hydrops, respiratory distress, osteochondritis, hemolytic anemia
25	34	1	64	4	512	Hepatosplenomegaly, thrombocytopenia, chorioretinitis

[a] RPR, Rapid Plasma Reagin test.

thaw cycles were avoided. Patients ranged in age from 1 to 66 days old. A total of 125 serum and 41 CSF samples were tested. Clinical divisions (infants symptomatic with congenital syphilis, asymptomatic infants at risk for syphilis, and infants of biologic false-positive mothers) were based on the information available to the physician who initially evaluated the infant. The clinical data on those infants with symptomatic illness or a reactive CSF-VDRL test are summarized in Table 1.

Western blot. Suspensions of solubilized *T. pallidum* proteins were prepared as previously reported (4) and subjected to discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis by using a 4% stacking gel over a 12% separating gel (15) in a miniaturized (7.0- by 8.5-cm finished gels) electrophoresis unit (Mini-Protean II System; Bio-Rad Laboratories, Richmond, Calif.). The separated proteins were then transferred to nitrocellulose paper by using an immunoblotting system (Mini-Transblot; Bio-Rad) of similar dimensions and a modification of the method of Towbin et al. (32). Electrophoretic transfer was performed at 100 V for 1 h. Low-molecular-weight standards (Bio-Rad) separated in an adjacent lane of each gel and transferred along with the treponemal proteins were stained with amido black and used to determine the efficiency of transfer and the relative molecular weights of *T. pallidum* antigens expressed in kilodaltons. Molecular weight designations of the separated proteins conformed to those identified in the consensus description (22).

Sheets of nitrocellulose were rinsed in phosphate-buffered saline with 0.05% Tween 20 (PBST) containing 1% fetal calf serum for 1 h to block nonspecific binding and then air dried. The sheets were then cut into 0.6-cm-wide strips for reacting with samples. Strips were reacted with a 2-ml volume of either serum or the IgM fraction at a 1:100 dilution in PBST or with CSF at a 1:50 dilution in sterile test tubes placed on a rocker. After overnight incubation, the strips were washed three times with 3 ml each of PBST and then reacted with a 2-ml volume of a mouse anti-human IgM monoclonal antibody (Clone MB-11; ICN Immunobiologicals, Lisle, Ill.) at a dilution of 1:2,000 in PBST for 2 h at room temperature. Strips were washed three times again and reacted with 2 ml of a 1:1,000 dilution of goat anti-mouse IgG conjugated with horseradish peroxidase (Tago, Inc., Burlingame, Calif.) for 2 h. After a final wash series, the strips were developed with 4-chloro-1-naphthol (HRP-Color Developer; Bio-Rad) according to the recommendations of the manufacturer. Reaction profiles were identified by comparison with standards and with silver-stained gels of *T. pallidum*.

IgM fractionation. To obtain IgM fractions free from IgG, 50 μl of the test serum was passed over a QuikSep IgM/IgG Isolation System II minicolumn (12; Isolab, Akron, Ohio), and fractions were collected and stored at −70°C. For Western blots, the IgM fractions were diluted to 1:100 final dilution. For the FTA-ABS (IgM) assay, the IgM fractions were reconcentrated approximately 10-fold by using centrifugal microconcentrators (Amicon Corp., Danvers, Mass.) so that IgM concentrations, as determined by radial immunodiffusion, were approximately equivalent to the original serum IgM concentration.

FTA-ABS (IgM) assay. Precleaned glass slides were rinsed

with sterile water and wiped to remove any excess oil film. *T. pallidum* antigen for the FTA-ABS (IgM) was prepared by using two different lots (FTA-ABS Antigen, BBL Microbiology Systems, Cockeysville, Md., and Difco Laboratories, Detroit, Mich.) and applied to matched pairs of slides according to the published recommendations for the standard FTA-ABS assay (33). Slides were then air dried, fixed in acetone, and stored at −20°C until used in the assay. Of each IgM fraction, 50 μl was diluted in 200 μl of PBS or FTA-ABS test sorbent (BBL) for a final dilution of 1:50 and layered over the test slides. Each sample was tested by using both antigen lots, both with and without sorbent. Slides were incubated in a moist chamber at 37°C for 30 min and rinsed twice for 5 min each in PBS and blotted dry. Fluorescein-conjugated anti-human IgM (μ-chain specific) (ICN) was diluted in PBS according to the product specifications and added to the test slides, which were again incubated for 30 min. After a second rinsing step, the slides were blotted dry and prepared for microscopic examination by using nonfluorescing FTA-ABS mounting fluid. All slides were examined with an Olympus fluorescence microscope within 4 h of testing, and the degree of fluorescence was graded from nonreactive to a reactivity of 4+. The assay was considered positive if either of the tested antigen lots yielded a 1+ or greater reaction.

Total CSF IgM determination. Since the Western blot is a qualitative rather than quantitative assay, the total amount of IgM in the CSF of 26 infants was determined by an enzyme-linked immunosorbent assay. Briefly, Immulon II flat-bottomed microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) were coated with 150 μl of mouse anti-human IgM (Sigma Chemical Co., St. Louis, Mo.) at a dilution of 1:8,000 in carbonate buffer, leaving peripheral wells empty. Plates were sealed and stored at 4°C until use. When CSF samples were ready for assay, plates were washed three times with PBST and nonspecific sites were blocked by flooding the plates with PBST containing 1% bovine serum albumin (radioimmunoassay grade) for 1 h. Plates were again washed three times, and 100-μl samples of serially diluted CSF (at 1:10, 1:20, and 1:60 initially; then at 1:100, 1:200, and 1:400 if further dilutions were necessary) in PBS containing 0.5% bovine serum albumin were added to triplicate wells and incubated for 2 h at room temperature. Following three washes with PBST, 100 μl of freshly prepared alkaline phosphatase-conjugated goat anti-human IgM at a 1:1,000 dilution in PBST containing 0.5% bovine serum albumin was added to all wells and incubated for 2 h at room temperature. Enzyme substrate was prepared by dissolving 5 mg of *p*-nitrophenylphosphate disodium (Sigma 104 Phosphatase Substrate; Sigma) in 5 ml of diethanolamine buffer. After a final wash sequence, 100 μl of substrate was added to all wells and the optical density at 405 nm was measured at 15, 30, and 45 min. Standard curves were generated by including dilutions of standard reference sera containing known amounts of IgM. Optical density was plotted against nanograms per milliliter of IgM in the reference sera, and amounts of IgM in the test samples were extrapolated from the linear portion of the standard curve.

Analysis of data. The sensitivity and specificity of the Western blot and FTA-ABS (IgM) assays were analyzed by using the two best-defined patient groups: those with symptomatic congenital syphilis (true-positive) and normal infants not at risk for disease (true-negative). Specificity for these serologic tests was defined as the percentage of unaffected individuals whose sera were nonreactive in the test in relation to the total number of unaffected individuals in the population tested. Sensitivity was defined as the percentage of affected patients whose sera were reactive in relation to the total number of affected patients in the population tested. The positive predictive values of both the IgM Western blot and the FTA-ABS (IgM) assays were calculated as indicators of their respective accuracies (10).

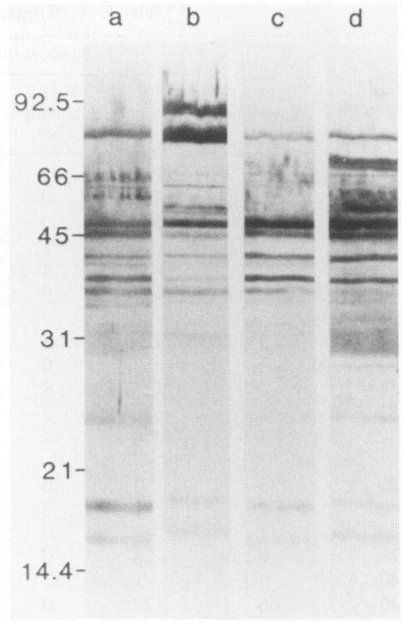

FIG. 1. IgM reactivity in Western blots of *T. pallidum* proteins. Lanes: a, pooled sera from adults with secondary syphilis; b to d, sera from infants with symptomatic congenital syphilis. Numbers to the left of the blots indicate relative molecular mass (kilodaltons).

RESULTS

Western blots. A typical pattern of IgM reactivity could be identified in the sera of infected infants by the Western blotting technique. In our assay, this profile consisted of IgM antibodies directed against the 47-, 17-, and 15.5-kilodalton (kDa) proteins of *T. pallidum* in all reactive samples and a variety of activities against the other low-molecular-weight proteins. IgM reactivity was frequently seen against the 45- and 37-kDa protein antigens and somewhat less often against the 42-, 34.5-, 31-, and 24-kDa antigens. The presence of at least five visually distinct IgM reactions (including specifically the 47-, 17-, and 15.5-kDa protein reactions) in serum samples was considered a positive or reactive blot. CSF was considered reactive if any IgM could be detected in the 47-to-15.5-kDa range. The 47-kDa antigen reaction was seen in all positive CSF samples, but fewer additional reactions were seen than in the serum samples.

Sera from 23 of 25 infants diagnosed with symptomatic congenital syphilis were positive for IgM reactivity against *T. pallidum* proteins by Western blot analysis. The two infants whose sera failed to show any IgM reactions both represented clinically confusing situations, one had been treated in utero and one had a questionable diagnosis (see Discussion). Figure 1 shows IgM Western blots from an adult with secondary syphilis (used as the positive control) and three infants with symptomatic congenital syphilis. None of 18 unexposed infants had reactive serum blots for *T. pallidum*. By using the previously stated definitions of sensitivity and specificity analysis of the Western blot assay,

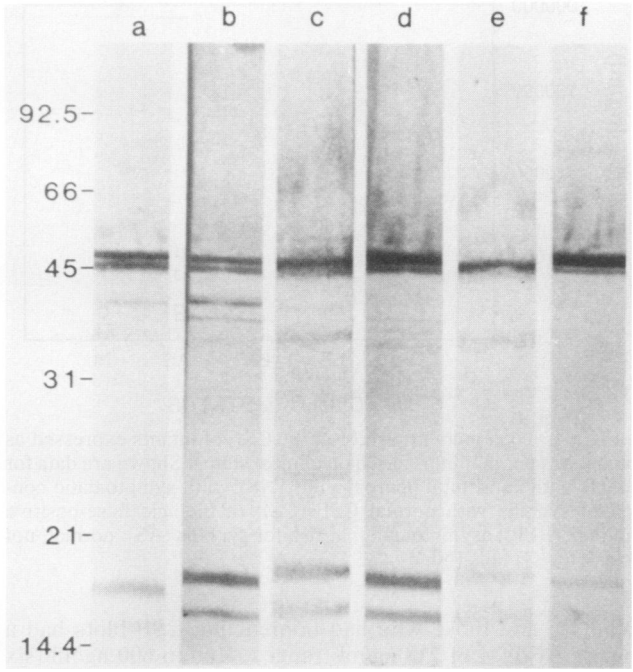

FIG. 2. IgM reactivity in whole sera and IgM fractions. Lanes a and b and c and d, paired serum and IgM fractions from two infants with symptomatic congenital syphilis; lanes e and f, serum and IgM fraction from an asymptomatic infant at risk for syphilis. Numbers at left indicate relative molecular mass (kilodaltons).

data for serum specimens yielded a sensitivity of 92.0%, which increased to 95.8% if the above-mentioned infant with questionable findings was excluded from the analysis, and a specificity of 100%. In comparison, the positive predictive value of the IgM Western blot was 95.3% (without excluding any infants).

When sera from infants with symptomatic congenital syphilis were fractionated in attempts to remove interference from maternal IgG and the IgM fractions were retested, 20 of the 23 samples remained reactive. The two infants whose sera were nonreactive initially remained nonreactive. Additionally, an infant whose serum showed strong IgM reactivity initially had fractionated serum studies done on a sample obtained after he required double-volume exchange transfusion on the second day of life, and these blots were nonreactive. When the IgM fraction data was analyzed, a sensitivity of 87.0% was observed, which increased to 95.2% if the previously mentioned infants were excluded. Figure 2 shows paired Western blots of the serum and IgM fractions from several infants tested.

Of 80 serum samples from asymptomatic infants at risk for congenital syphilis (Table 2), 16 exhibited IgM directed against *T. pallidum*. The patterns of IgM reactivity were similar for symptomatic and asymptomatic infants. There were, however, five serum samples from asymptomatic infants at risk for congenital syphilis which were characterized by very faint staining of only a few IgM reactions when the blots were developed. These five samples all yielded nonreactive blots when they were fractionated and the IgM fractions were retested.

Of the 17 infants with symptomatic congenital syphilis who had IgM Western blots of their CSF done, 14 exhibited reactivity. Figure 3 shows examples of CSF blots. Of these

TABLE 2. IgM reactivity in Western blots of *T. pallidum*

Clinical status of infant	No. positive/no. tested[a]		
	Serum	IgM fraction	CSF
Symptomatic congenital syphilis	23/25	20/23	14/17
Asymptomatic, at risk	16/80	8/16[b]	1/23
Biologic false-positive mother	0/2	ND[c]	0/1
Normal	0/18	ND	ND

[a] One infant whose serum, IgM fraction, and CSF failed to react with blots was born to a mother treated 1 month before delivery. Another, who had a very low birth weight and multiple medical problems related to prematurity, was born to a mother with untreated syphilis but exhibited no *T. pallidum*-directed IgM. One infant with initially reactive serum required double-volume exchange transfusion and subsequently had nonreactive CSF and IgM fraction blots.

[b] Of these 16 infants, 13 had exhibited reactive serum blots.

[c] ND, Not done.

14 infants, 12 had reactive CSF-VDRL tests (Table 3). Five infants, one of whom was completely asymptomatic, had nonreactive CSF-VDRL tests but showed IgM reactivity on Western blot analysis of their CSF. Two infants with reactive CSF-VDRL tests had nonreactive CSF blots, although both presented with some atypical features. Reactions of *T. pallidum*-directed IgM in the CSF were similar to those seen in the serum and IgM-enhanced fractions, but there were few reactions other than that corresponding to the 47-kDa protein. Using the previously stated definitions, analysis of the Western blot data for the CSF assays compared with the CSF-VDRL test yielded a sensitivity of 83.3% and a specificity of 82.7%.

FTA-ABS (IgM). Of the 21 infants with symptomatic congenital syphilis who had further studies done, 16 had reactive FTA-ABS (IgM) tests compared with 19 who had reactive serum blots (18 of whom had reactive IgM fraction Western blots) (Table 4). Two patients had both negative

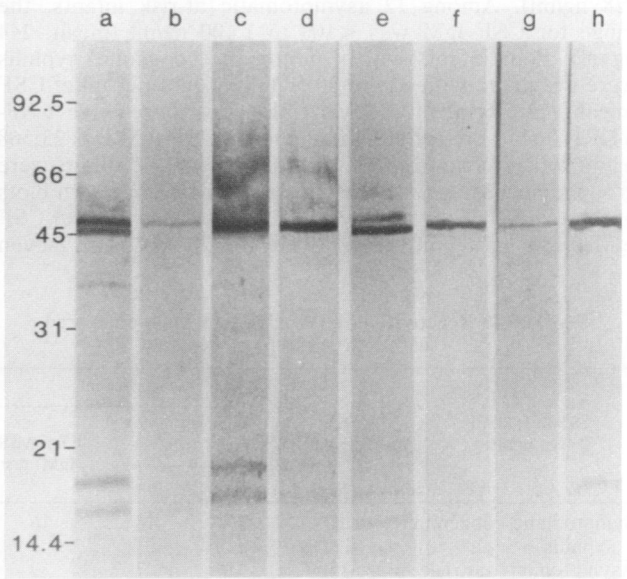

FIG. 3. CSF IgM reactivity in Western blots of *T. pallidum*. Lanes a and b and c and d, paired sera and CSF from two infants with symptomatic congenital syphilis; lanes e to g, CSF from three other infants with symptomatic infection; lane h, CSF from an asymptomatic infant at risk for syphilis. Numbers at left indicate relative molecular mass (kilodaltons).

TABLE 3. IgM Western blot of CSF compared with CSF-VDRL test for infants with presumed congenital syphilis

CSF Western blot	No. in CSF-VDRL test	
	Reactive	Nonreactive
Reactive	10	5[a]
Nonreactive	2[b]	24

[a] One infant with a nonreactive VDRL test and reactive CSF blot was otherwise asymptomatic.
[b] Both infants with reactive VDRL tests and nonreactive CSF blots were atypical (see text).

IgM Western blots and nonreactive FTA-ABS (IgM) sera. Selection of sera of infants for FTA-ABS (IgM) testing was limited to those patients for whom adequate sample volumes existed. This constraint unfortunately resulted in the group of asymptomatic, at-risk infants being weighted toward those whose sera had exhibited reactive Western blots. Of the 15 asymptomatic infants tested, 9 had reactive FTA-ABS (IgM) tests, while 11 had reactive serum blots and 7 had reactive IgM fraction blots. Thus, the serum IgM Western blot detected 90.5% of the symptomatic infants in this phase of the study compared with 76.2% detected by the FTA-ABS (IgM) assay. Of the nine normal infants and three nonreactive adults (negative controls) tested, serum samples of four infants showed reactivity in the FTA-ABS (IgM) test, resulting in a false-positive rate of 33.3%. None were reactive in IgM Western blots. The positive predictive value of the FTA-ABS (IgM) test was 72.7%.

Total CSF IgM determination. While it was not feasible to quantitate the amount of *T. pallidum*-directed IgM in the CSF of infants thought to be at risk for neurosyphilis, it was possible to measure the total amount of CSF IgM. Among 10 infants not at risk for syphilis, total CSF IgM ranged from <100 to 890 ng/ml, with a mean of 276 ng/ml. Of three infants of biologic false-positive mothers, the range for CSF IgM was from <100 ng of IgM per ml of CSF to 185 ng/ml (mean, 128 ng/ml). Among 12 asymptomatic at-risk infants, the range for CSF IgM was <100 to 1,100 ng/ml (mean, 244 ng/ml). Four infants with symptomatic congenital syphilis were evaluated with 228 to 30,500 ng of IgM per ml of CSF (mean, 13,546 ng/ml). Seven infants with reactive CSF-VDRL tests were tested, and the mean CSF IgM was 23,568 ng/ml with a range from 135 to 120,000 ng/ml. If infants were grouped according to the results of their IgM Western blots of the CSF, those with reactive CSF blots had a mean CSF IgM of 30,805 ng/ml (range, 2,116 to 120,000 ng/ml [seven infants]), and those who had nonreactive CSF blots had a mean CSF IgM of 218 ng/ml (range, <100 to 600 ng/ml [six infants]). Figure 4 shows these data plotted as nanograms per milliliter of IgM in the CSF according to clinical status.

TABLE 4. Reactivity in FTA-ABS (IgM) test compared with IgM Western blots

Clinical status of infant	No. tested	Reactivity in[a]:		
		Western blot with:		FTA-ABS (IgM) test
		Serum	IgM	
Symptomatic congenital syphilis	21	19	18	16
Asymptomatic, at risk	15	11	7	9
Normal	9[b]	0	0	4

[a] Two infants diagnosed with congenital syphilis had both nonreactive blots and FTA-ABS (IgM) results. Another had reactive serum and then a nonreactive IgM fraction blot and FTA-ABS (IgM) result following exchange transfusion.
[b] Sera from three adults were also nonreactive in the FTA-ABS (IgM) test.

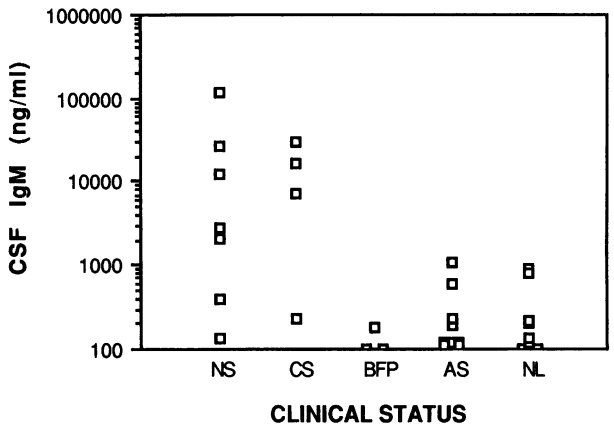

FIG. 4. Total amount of IgM in the CSF of infants expressed as nanograms per milliliter, based on clinical status. Shown are data for infants with congenital neurosyphilis (NS); with symptomatic congenital syphilis with normal CSF (CS); of biologic false-positive mothers (BFP); asymptomatic, at risk for syphilis (AS); normal, not at risk for syphilis (NL).

DISCUSSION

The results of this study confirm and extend earlier work done in our laboratory and other laboratories suggesting that Western blot analysis of IgM antibodies directed against *T. pallidum* can confirm congenital infection even in asymptomatic infants at risk for the disease. The IgM and IgG antibody responses in adult patients in all stages of syphilis, both before and after treatment, have been described elsewhere (1, 3), although no pattern is diagnostic of a particular stage. IgM antibodies directed against several of the lower-molecular-weight proteins of *T. pallidum* could be reliably identified in the sera and CSF of infants with symptomatic congenital syphilis and were uniformly absent in infants with no exposure to syphilis. Reactions with the 47-, 17-, and 15.5-kDa proteins were found in all reactive blots, together with a variety of other reactions, including those corresponding to the 45-, 42-, 37-, 34.5- 31-, and 24-kDa proteins. These data are in agreement with the preliminary study by Dobson et al. from this laboratory (8) and the later work of Sanchez et al. (25), which indicate that the 47-kDa protein is one of the major immunogens in neonatal IgM antibody production in congenital syphilis. Antigenic cross-reactivity between *T. pallidum* and other pathogenic spirochetes has been reported (2), although the present consensus is that the 47-kDa antigen is one of the major immunodominant (22), pathogen-specific (21) molecules. In our laboratory, serum samples from six patients with diagnosed Lyme disease showed some IgM reactivity to the 45- and 37-kDa antigens but none to the 47-kDa or the other lower-molecular-weight (<34.5-kDa) proteins (data not shown).

Serum samples from two infants diagnosed with congenital syphilis failed to show IgM reactivity in blots with either serum or IgM. Of these, one had been adequately treated in utero 1 month before delivery and was asymptomatic at birth but had a reactive CSF-VDRL test and therefore met the conventional criteria for congenital neurosyphilis. The second infant was delivered at 26 weeks of gestation to a mother

with untreated secondary syphilis; although the infant had multiple medical problems, it was unclear whether her nonspecific symptoms were related to congenital syphilis or were the consequences of extreme prematurity, and the infant was treated for presumed syphilis. Another infant with symptoms very suggestive of congenital syphilis initially had a reactive serum blot but required double-volume exchange transfusion two times on day 2 of life for hyperbilirubinemia, and subsequent samples used in the fractionation step were nonreactive, as was CSF obtained after several days. The latter two infants were excluded from the data analysis (see Results). Of the 80 asymptomatic infants thought to be at risk for congenital syphilis, 16 (20%) had reactive serum IgM Western blots, a value within the expected range for development of congenital syphilis on the basis of previous epidemiologic studies (20).

Criticism of the FTA-ABS (IgM) test includes lack of standardization between laboratories, lack of reproducibility within a laboratory, a false-negative rate as high as 35% in certain patient groups, a false-positive rate of 10%, and inadequate evaluation in asymptomatic infants with reactive STS (14). Certain of these problems appear to originate with selection of the fluorescein-conjugated second antibody used for detection. Indeed, in our laboratory over a period of 10 years, we have found that individual lots from different sources require considerable evaluation prior to use (R. E. Baughn, unpublished observations). In the present study, we chose to use a μ-chain-specific second antibody which failed to exhibit cross-reactivity with affinity-purified IgG from pooled syphilitic sera. Despite considerable preliminary quality assurance of this second antibody prior to use (data not shown), both the false-negative and positive predictive values of the FTA-ABS IgM test in the present study were at best disappointing.

With regard to the false-positive rate of the FTA-ABS (IgM) test, Reimer et al. (24) implicated RF as the entity responsible, because the majority of infants (26 of 27 in his review) with congenital syphilis had RF. Despite the potential interference of RF in the FTA-ABS (IgM) test, our results are in agreement with two recent studies (8, 25), which have suggested that rigorous removal of RF is not necessary prior to Western blot analysis. Our study made use of a commercially available ion-exchange chromatography column which, on the basis of quantitative radial immunodiffusion studies in our laboratory (data not shown), removes 90% of the IgG. In most cases, the IgM reactivity on Western blots of the fractionated sera was enhanced. However, five serum samples which exhibited very faint staining of a small number of IgM bands converted to nonreactive when the IgM fraction of serum was retested. Clinically, there were no findings to distinguish those asymptomatic patients whose original Western blot results might best be called equivocal or indeterminate from other asymptomatic infants with reactive blots, but the qualities of the blots were noticeably different.

CSF-VDRL reactivity has traditionally been accepted as indicative of central nervous system (CNS) disease. Recent studies have, however, documented that in adults, false-negative reactions may occur with this test and results may not correlate with either CNS symptoms or abnormal CSF protein levels or cell counts (29, 34, 35). It has been shown that maternal IgG can diffuse into the CSF in neonates (31), thereby potentially increasing the likelihood of a false-positive CSF-VDRL test. Our results indicate that the Western blot assay is probably more sensitive than the conventional CSF-VDRL test in detecting CNS involvement in infants with congenital syphilis, but again correlation with neurologic symptoms or abnormal CSF parameters was poor, and it was not possible to distinguish locally produced IgM from that which could have represented contamination from serum. None of the infants in our study had specific neurologic findings, and only two were described as irritable, while only one had abnormal CSF findings. In our group of infants, two with reactive CSF-VDRL tests had nonreactive CSF IgM blots. One of these was the previously mentioned infant requiring double-volume exchange transfusion. The other infant was completely asymptomatic and had a low-titer STS and a maternal history of treatment but had a reported CSF-VDRL titer of 1:32, with normal CSF glucose, protein, and cell count. Whereas these results seemed inconsistent, we were unable to confirm the CSF-VDRL results in another laboratory because of insufficient quantities of CSF.

Efforts to quantitate the levels of CSF IgM in patients in different clinical and epidemiological categories revealed that total amounts of IgM in the CSF varied widely but were elevated in most infants with symptomatic congenital syphilis, both with and without reactive CSF-VDRL test results, compared with asymptomatic infants and infants not at risk for syphilis. This suggests that there was increased local IgM production or gross distortion of the blood-brain barrier in some infants with syphilis in whom the CSF-VDRL test was nonreactive. Other infectious processes have been shown to both increase local synthesis of IgM in the CNS and damage the blood-brain barrier in adults (11, 28), but little information is available about the production of IgM in the CSF of infants. Less variation was seen in the CSF IgM ranges if the patients were grouped according to their CSF IgM blot reactivity, but the number of infants who had both studies done was small. These data again lead to the conclusion that the CSF IgM blot may more accurately detect even asymptomatic infants producing *T. pallidum*-directed IgM within the CNS. The detection of asymptomatic CNS involvement has important ramifications in treatment, since the CDC now recommends a regimen adequate for treating neurosyphilis in all infants with confirmed or compatible congenital syphilis regardless of the CSF-VDRL results (6).

The miniaturized Western blot assay for *T. pallidum*-directed IgM has several practical advantages over the conventional assay. The smaller gels and blots are generally less cumbersome to prepare and manipulate. Smaller quantities of treponemal antigen suspension are required, an important consideration since preparation of the solubilized proteins requires propagation of the organism in rabbits and is both expensive and tedious. The use of the monoclonal anti-IgM antibody also improved the results of the assay. With its use, there was no cross-reactivity with IgG antibodies detected even when the IgG fractions of several reactive sera were tested in a Western blot (data not shown). Nitrocellulose strips can be prepared in advance and then reacted with serum and CSF samples as they become available, since storage of blots at −20°C for up to 3 months does not appear to result in diminution or loss of reaction patterns. More importantly, by using preprepared strips, the sample testing process could easily be performed within 8 to 24 h, expediting therapeutic decisions. Compared with the rather laborious FTA-ABS (IgM) test, blots are easily interpreted without the distinct disadvantage of subjective scoring. Thus, IgM Western blotting appears to be an excellent tool as a confirmatory test for congenital syphilis, although more work has yet to be done to better define the maternal-infant antibody interactions, their role in pathogenesis of the disease, and their utility in diagnostic studies.

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