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Prenatal Diagnosis of Congenital Cytomegalovirus Infection

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We report here the results of a study on the prenatal diagnosis of congenital cytomegalovirus (CMV) infection. The study was carried out by both PCR and virus isolation from amniotic fluid (AF) for 82 pregnant women at risk of transmitting CMV for the detection of (i) seroconversion to CMV immunoglobulin G (IgG) positivity during the first trimester of pregnancy, (ii) symptomatic CMV infection in the mother during the first trimester of pregnancy or intrauterine growth retardation detected by ultrasound or abnormal ultrasonographic findings suggestive of fetal infections, and (iii) seropositivity for CMV-specific IgM. For 50 women, fetal blood (FB) was also obtained and tests for antigenemia and PCR were performed. The results indicate that AF is better than FB for the prenatal diagnosis of CMV infection. PCR with AF has a sensitivity (SNS) of 100%, a specificity (SPE) of 83.3%, a positive predictive value (PPV) of 40%, and a negative predictive value (NPV) of 100%; rapid virus isolation with the same material has an SNS of 50%, an SPE of 100%, a PPV of 100%, and an NPV of 94.7%. Fewer than 10% of the women positive for IgM by enzyme immunoassay (EIA) had a congenitally infected fetus or newborn infant. When EIA IgM positivity was confirmed by Western blotting (WB) and the WB profile was considered, the percent transmission detected among women with an "at-risk" profile was higher than that observed among IgM-positive women and was the same as that among women who seroconverted during the first trimester of pregnancy (transmission rates of 29 and 25%, respectively).

Congenital cytomegalovirus (CMV) infection is the leading cause of congenital viral infection in developed countries, occurring with a stable incidence in 0.4 to 2.2% of all live births (1, 3). Intrauterine infection occurs in up to 50% of pregnancies following primary maternal infection, and 10% of the infected infants are symptomatic at birth (31). In addition, 5 to 15% of asymptomatic babies are at risk of developing sequelae, particularly sensorineural hearing loss (30). Reactivated maternal infections involve mostly minor consequences for the offspring (8).

The natural history of intrauterine CMV infection is not well understood, but it is clear that fetuses are irreversibly damaged by the virus before delivery. Those infants would not derive much benefit from postnatal therapy, but if infected fetuses could be detected before this irreversible stage has been reached, treatment in utero (when available) might have a significant effect on the course of disease (35).

It has repeatedly been shown that isolation of virus from amniotic fluid (AF) is effective in differentiating uninfected from infected fetuses (12, 14, 15, 17, 25). However, although prenatal diagnosis of congenital CMV infection was first reported by Davis et al. in 1971 (5), the number of reported cases is still low (13, 29). Questions concerning the sensitivity of AF culture persist, because a few false-negative results have been reported in the literature (4, 7, 27). PCR has also been applied to CMV genome detection in AF from 26 pregnant women and has been shown to have a positive predictive value of 100% and a negative predictive value of 81% (29). In this study neither PCR nor virus isolation could detect all cases of congenital CMV infection prenatally.

Besides the small numbers of cases of CMV infection diagnosed prenatally and the consequent limited experience with prenatal diagnosis, another important problem hampers this diagnostic aspect of CMV infection: it is difficult to determine which women should be enrolled in prenatal diagnostic programs. In fact, only pregnant women undergoing a primary CMV infection should be enrolled in prenatal diagnostic programs, and when seroconversion is not detected, the diagnosis of a primary infection is still problematic (24, 33).

We report here the results of a study on the prenatal diagnosis of CMV infection carried out by both PCR and virus isolation from AF for 82 pregnant women defined as being at risk of transmitting CMV. For 50 women fetal blood (FB) was also obtained and tests for antigenemia and PCR were performed.

The results obtained indicate that AF is a more suitable material than FB for prenatal diagnosis. Furthermore, neither PCR nor virus isolation should be used alone. However, the combination of the two methods gives a sensitivity and a specificity of 100% each. Finally, Western blotting (WB) may be useful in determining which women should be enrolled in prenatal diagnostic programs.

MATERIALS AND METHODS

Cells and virus. CMV AD169 was propagated in human embryo fibroblasts (HELs) by standard methods (18).

Pregnant women. Between January 1994 and May 1998, 82 pregnant women who attended the Center for Prenatal Diagnosis of the II Department of Obstetrics and Gynecology of the University of Bologna were enrolled in a program for the prenatal diagnosis of CMV infection for the following reasons: (i) documented seroconversion to positivity for CMV from 3 months before conception to the end of the first trimester of pregnancy (16 women); (ii) CMV symptoms (increase in liver enzyme levels and/or fever) and presence of CMV immunoglobulin M (IgM) (2 women); (iii) intrauterine growth retardation as detected by ultrasound (1 woman); (iv) abnormal ultrasonographic findings suggestive of fetal infections (2 women); and (v) the presence of CMV-specific IgM (61 women).

Twenty pregnant women at no risk of fetal CMV transmission but from whom AF and FB were taken for fetal karyotype assessment constituted the control group.

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TABLE 1. Prenatal diagnosis carried out by PCR and antigenemia testing with FB and by PCR and rapid virus isolation with AF in relation to outcome for fetus or newborn for pregnant women at risk of transmitting a CMV infection and control population[a]

Group	FB					AF				
	Total no. of specimens	No. of specimens positive by PCR	No. of specimens positive by AG testing	Infected fetus or newborn		Total no. of specimens	No. of specimens positive by PCR	No. of specimens positive by SV	Infected fetus or newborn	
				Fetus/ newborn[b]	Total no.				Fetus/ newborn	Total no.
Seroconversion for CMV IgG	10	2	1	0/1	1	16	9	1	1/3	4
IgM positivity by ELISA	39	10	1	2/3	5	61	14	4	3/3	6
Clinical symptoms (mother/fetus)	1	0	0	0	0	5	4	1	1/1	2
Total	50	12	2	2/4	6	82	25	5	5/7	12
Uninfected (EIA IgM negative)	11	0	0	0	0	20	0	0	0	0

[a] AG, antigenemia; SV, shell vial assay.
[b] Number of infected fetuses/number of infected newborns.

Amniocentesis and cordocentesis. Twenty milliliters of AF was collected by transabdominal amniocentesis under continuous ultrasound guidance. Two milliliters of FB was collected by funipuncture under ultrasound guidance by using a biopsy guide and a 20- and 25-gauge needle combination as described by Bovicelli et al. (2).

Amniocentesis and cordocentesis were performed at 21 to 22 weeks of gestation, with informed consent obtained from all women.

Determination of a congenital CMV infection in newborns. A congenital CMV infection in a newborn was determined by isolation of CMV from urine or saliva during the first week of life.

CMV serology. (i) Conventional enzyme immunoassay (EIA). The evaluation of anti-CMV IgG was carried out with a commercial kit (Enzygnost anti-CMV/ IgG EIA alpha method; Behring AG, Marburg, Germany). Plates were read on a micro-EIA automatic reader (Behring AG). The evaluation of anti-CMV IgM was performed with the Enzygnost anti-CMV/IgM kit (Behring AG). Both kits were used, and the results were interpreted as suggested by the manufacturer.

(ii) WB. Protein extracts from purified viral particles (Towne strain) were run in a 9% acrylamide gel, and the electrophoretically separated polypeptides were then transferred to nitrocellulose paper. At the bottom of the viral blot were three recombinant proteins containing significant portions of ppUL32 (p150), ppUL44 (p52), and ppUL57 (p130) as well as two additional control proteins (the CKS protein was added as a negative control to monitor the presence in serum of IgM to the bacterial portion of the fusion protein and human μ chain as a positive control to monitor the reaction of the conjugate to human IgM). This WB was described in detail in a previous report (21).

Infection of cells, virus purification, protein extraction, blotting, and detection of immune reaction with sera were done as previously described in detail (19).

Virological detection of CMV. (i) CMV isolation. The shell vial procedure (11) was used for CMV isolation from urine and saliva. The cells were fixed 24 to 48 h after inoculation and were stained by an indirect immunofluorescence assay with a monoclonal antibody reacting with the CMV IE1 and EA gene product (E13 + 2A2; Argene, Varilhes, France).

(ii) Antigenemia. The presence of CMV pp65 (ppUL83) in polymorphonuclear leukocytes (PMNLs) was determined as originally described by van der Biji et al. (32) and modified by Revello et al. (28) by using a CMV pp65-specific pool of two monoclonal antibodies (1C3 and AYM-1; Argene) in indirect immunofluorescence tests.

(iii) Determination of the presence of CMV genome by PCR. CMV DNA was individually extracted from three to six aliquots of AF (100 μl each) with an IsoQuick Nucleic Acid Extraction Kit (Orca Research, Bothell, Wash.) and was resuspended in 50 μl of RNase-free water. Aliquots of 5×10^5 PMNLs were resuspended in 100 μl of PCR buffer (KCl, 50 mM; Tris-HCl, 10 mM [pH 8.3]; $MgCl_2$, 2 mM; gelatin, 0.01%), with nonionic detergents and proteinase K. Samples were incubated at 60°C for 1 h and then at 95°C for 20 min to inactivate the proteinase. A total of 30 μl of each sample of PMNLs and DNA extracted from each aliquot of AF were added to 20 μl of a reaction buffer containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2 mM $MgCl_2$, the four deoxynucleotide triphosphates at a concentration of 0.2 mM each, 50 pmol of each primer, and 1.25 U of Amplitaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.). The primer pair of Demmler et al. (6) targeting a 435-bp sequence within the major immediate-early antigen of the CMV Towne strain was used (DIGENE Diagnostics, Beltsville, Md.), with one primer 5′ labelled with biotin. Samples were denatured at 94°C for 5 min and subjected to 40 amplification cycles. PCR products were denatured and hybridized with a CMV-specific RNA probe. RNA-DNA hybrids were transferred to streptavidin-coated capture plates. Alkaline phosphatase-labelled RNA-DNA hybrid antibody was added, and bound antibody was detected by using p-nitrophenylphenol. The optical density at 405 nm was read with a microplate reader at 1, 18, and 24 h after substrate addition. The color intensity was proportional to the amount of captured RNA-DNA hybrids, with maximum assay sensitivity achieved 24 h after substrate addition.

The cutoff was calculated as twice the mean for the negative controls (a minimum of four per assay) plus 0.08 (Digene SHARP Signal System; DIGENE Diagnostics). AF was considered positive if at least one of the aliquots was positive. To prevent carryover of amplified DNA sequences and false-positive reactions, samples were prepared under a biosafety hood that was located in a room separate from that in which PCR was performed. Separate sets of supplies and positive pipetting devices were used for sample preparation and to set up the reactions. All other recommended precautions for PCR were followed.

RESULTS

As indicated in Table 1, we monitored 16 pregnant women who seroconverted to anti-CMV IgG positivity during the first trimester of pregnancy. For 9 of 16 (56.3%) women, PCR detected the viral genome in the AF, while in only 1 woman was PCR positivity correlated with viral isolation. Eight of nine women decided to continue their pregnancies, and the virus was isolated from the urine of three newborns. Among these three congenitally infected newborns one suffered from a mild case of CMV-related hepatitis and two were asymptomatic. One woman decided to interrupt her pregnancy. Her fetus showed CMV inclusions in the liver and lung (data not shown).

For 14 (22.9%) of the 61 women in whom CMV-specific IgM was detected by EIA, the viral genome was detected in the AF by PCR. For four women PCR positivity was correlated with isolation of virus from AF. Eleven of 14 women decided to continue their pregnancies, and the virus was isolated from the urine of three of the offspring. Among these three congenitally infected newborns, one suffered from monolateral ventriculomegaly and two were asymptomatic (data not shown). Three women decided to interrupt their pregnancies. In two fetuses CMV inclusions in multiple organs were detected, and one fetus showed CMV polyvisceritis (data not shown).

For two mothers with symptomatic CMV infection, the viral genome was detected in the AF, but this was not correlated with virus isolation or with evidence of congenital infection in the two newborns. For one woman for whom prenatal diagnosis was carried out on the basis of the detection of fetal growth retardation, no CMV was found in the AF and no evidence of congenital infection was detected in the newborn. The infection in this patient was later diagnosed to be due to a parvovirus infection (data not shown).

We also tested by PCR and virus isolation 20 AF specimens that were obtained for fetal karyotype assessment. In no case did PCR or virus isolation give a positive result. No congenitally infected newborns were found in this group.

At the beginning of our study we also performed prenatal diagnostic tests with FB taken by cordocentesis. Antigenemia tests and PCR were carried out with 50 FB samples from women at risk of transmitting the infection and 11 samples

TABLE 2. Sensitivity, specificity, PPV, and NPV of antigenemia tests and PCR with PMNLs from FB and of PCR and rapid virus isolation from the AF with regard to the detection of a CMV-infected fetus or newborn

Material	Test	Result	No. of fetuses or newborns		Sensitivity[a]	Specificity[b]	PPV[c]	NPV[d]
			Infected	Uninfected				
FB	PCR	Positive	4	8	66.6	85.5	33.3	96
		Negative	2	47				
	Test for antigenemia	Positive	1	1	16	98.2	50	91.5
		Negative	5	54				
AF	PCR	Positive	12	15	100	83.3	48	100
		Negative	0	75				
	Shell vial assay	Positive	6	0	50	100	100	93.8
		Negative	6	90				

[a] (Number of true positives/total number of fetuses or newborns infected) × 100.
[b] (Number of true negatives/total number of fetuses or newborns uninfected) × 100.
[c] (Number of true positives/number of true positives + number of false positives) × 100.
[d] (Number of true negatives/number of false negatives + number of true negatives) × 100.

obtained for fetal karyotype assessment. As indicated in Table 1, PCR with FB detected the viral genome in the fetuses of 12 women. For two women this result was accompanied by a positive antigenemia test result. For only one antigenemia-positive woman did we document a CMV-positive newborn. Among the 12 women whose fetuses were CMV positive by PCR with FB, two fetuses and four newborns had signs of CMV infections. We also tested 11 FB samples that were obtained for fetal karyotype assessment. In no case did PCR or antigenemia tests give a positive result.

From the data presented in Table 1, we calculated the sensitivities, specificities, positive predictive values (PPVs), and negative predictive values (NPVs) of tests for antigenemia and PCR with PMNLs from FB and of PCR and rapid virus isolation from the amniotic fluid with regard to the detection of a CMV-infected fetus or newborn (Table 2). AF seems a better material than FB for predicting an infected fetus or newborn. This evidence, together with the fact that cordocentesis is a more invasive procedure than amniocentesis, led us to continue our study with AF only. Therefore, the subsequent prenatal diagnoses were carried out by PCR and rapid virus isolation with AF only. As indicated in Table 2, while PCR detected all congenital infections, its specificity was 83% and its PPV was 48%. On the other hand, virus isolation missed 50% of the infections in fetuses or newborns.

Commercially available EIAs for anti-CMV IgM detection have poor reliabilities. This is a critical point in terms of deciding which women should be enrolled in prenatal diagnostic programs; thus, we investigated whether WB can be of any help in this regard. As indicated in Table 3, among 61 women who were judged to be IgM positive by EIA, 45 (73.7%) were confirmed to be positive by WB. Interestingly, all six cases of congenital CMV infections occurred in those pregnant women whose serum showed the presence of anti-CMV IgM as confirmed by WB. In none of the 16 women whose IgM positivity was not confirmed by WB was the viral genome found in the AF by PCR.

Since particular WB profiles are mainly linked to primary infections (19, 20), we divided sera confirmed to be positive by WB into three groups on the basis of their profiles. In particular, we included in the group of sera from primarily infected women those with a strong IgM reactivity to p52 and/or p65 in the presence of low amounts (or complete absence) of IgM to

vp150. Among this group, those with a strong reactivity with more than four bands were also included (23). Furthermore, we included in the group of secondarily infected women those with an IgM reactivity to vp150 and vp38 only (16). Finally, we included women (third group) with an undefined WB profile.

As shown in Table 3, 21 serum samples were from women with an "at-risk" profile (46.6%), while 8 serum samples were from women with non-primary infections (20%) and 16 serum samples were from women with an undetermined profile (35.5%).

Interestingly, all six cases of congenital CMV infections occurred among those pregnant women whose serum showed a profile indicative of a recent primary infection or a high number of reactive bands.

However, in one of the eight women with a non-primary infection and in two of those with an undetermined type of infection, PCR detected the viral genome in the AF. Virus was not isolated from any of these women, and no infected fetuses or newborns were observed.

From the data presented in Table 3, we calculated the sensitivity, specificity, PPV, and NPV of maternal serology with regard to the detection of a CMV-infected fetus or newborn (Table 4). While all three serological approaches had 100% sensitivity and 100% NPV, the specificity and the PPV were

TABLE 3. Prenatal diagnosis and congenital infections in pregnant women with CMV-specific IgM as determined by EIA and WB

Group	No. of women				
	IgM-positive sera by EIA		IgM-positive sera by WB by type of infection		
	WB positive (n = 45)	WB negative (n = 16)	Primary (n = 21)	Secondary (n = 8)	ND[a] (n = 16)
Prenatal diagnosis with AF					
PCR positive	14	0	11	1	2
Virus isolation positive	4	0	4	0	0
Congenital infections	6	0	6	0	0

[a] ND, not determined.

TABLE 4. Sensitivity, specificity, PPV, and NPV of maternal serology with regard to the detection of a CMV-infected fetus or newborn

Test	Result	No. of fetuses or newborns		Sensitivity[a]	Specificity[b]	PPV[c]	NPV[d]
		Infected	Uninfected				
Seroconversion	Positive	4	12	100	62.5	25	100
	Negative	0	20				
IgM by ELISA	Positive	6	55	100	26.6	9.8	100
	Negative	0	20				
IgM WB profile	Positive	6	15	100	72.7	28.5	100
	Negative	0	40				

[a] (Number of true positives/total number of fetuses or newborns infected) × 100.
[b] (Number of true negatives/total number of fetuses or newborns uninfected) × 100.
[c] (Number of true positives/number of true positives + number of false positives) × 100.
[d] (Number of true negatives/number of false negatives + number of true negatives) × 100.

very low for the enzyme-linked immunosorbent assay (ELISA) for IgM and much higher for IgG seroconversion and the WB profile.

DISCUSSION

This paper reports the results of a study on the prenatal diagnosis of congenital CMV infection mainly carried out with pregnant women who seroconverted during pregnancy or who had CMV-specific IgM. For only five women were prenatal diagnostic procedures carried out on the basis of clinical suggestions.

At the beginning of our study we obtained from pregnant women both AF (with which we performed a shell vial assay and PCR) and FB (with which we performed PCR and tests for antigenemia). When we had monitored 50 women we compared the results obtained with AF and FB and realized that the results obtained by both PCR and tests for antigenemia of FB gave PPVs and NPVs for congenital infection poorer than those obtained with AF. For this reason and because cordocentesis is a more invasive procedure than amniocentesis (10), we decided to continue our study with AF alone. AF was obtained from 102 women (82 women at risk of transmitting CMV infection and 20 women who were used as a control group).

Interestingly, PCR detected viral DNA in the AF from 27 of 82 women, and in only 12 of them did we document a congenital infection in newborns at birth (PPV, 48%). This is probably due to the high sensitivity of the procedure (100%), which detects a viral load so low as to be cleared by the defenses of the mother or fetus. Quantitative PCR is in progress to verify this hypothesis.

On the basis of this observation, the term "rate of intrauterine transmission of CMV," which is usually applied to the percentage of CMV-infected newborns whose infections are detected at birth, should instead be applied to the percentage of AF-positive specimens, which is higher than the actual percentage of infected newborns whose infections are detected at birth.

Because of these findings it seems reasonable to conclude that PCR cannot be used alone for prenatal diagnosis of CMV infection but should be complemented with virus isolation, which has a specificity and a PPV of 100%, although it suffers from poor sensitivity.

The proportion of congenital infections detected in pregnant women who seroconverted during the first trimester of pregnancy is 25%, and 50% of the infected fetuses or newborns are affected. These results are in agreement with those of many other published reports (9, 26, 31).

Furthermore, the proportion of congenital infections detected in pregnant women who had CMV-specific IgM, as detected by ELISA, during the first trimester of pregnancy is 9.8%. This result indicates that more than 90% of prenatal diagnostic tests that result in a diagnosis on the basis of IgM positivity by ELISA only are unnecessary. When the IgM positivity by ELISA is confirmed by WB, the correlation with transmission is not significantly different. On the contrary, if the WB profile is taken into consideration, the rate of transmission for women with an at-risk profile is the same as that for women who seroconverted during the first trimester of pregnancy (rates of transmission, 29 and 25%, respectively). An at-risk profile means that IgM is preferentially directed to rp52 and/or vp65 or that serum IgM reacts with more than four bands (19, 20, 23).

By using this criterion, only one-third of IgM-positive serum samples (21 of 61 serum samples) were judged to be from women at risk of transmitting the infection congenitally, and this would avoid the need for prenatal diagnosis for two-thirds of the women at risk. Another possibility for deciding which women should be enrolled in prenatal diagnostic programs would be IgG avidity determination (22). A study aimed at determining the usefulness of this procedure is under way.

In conclusion, congenital CMV infection is a major health problem that should be approached on the basis of which women should be enrolled in prenatal diagnostic programs, which clinical specimens should be tested, and which laboratory procedures should be adopted for the diagnosis of congenital CMV transmission or infection.

However, a major drawback is that at present there is not enough evidence to predict the outcome for CMV-infected fetuses. The natural history of intrauterine CMV infection is not well understood, but it is clear that some fetuses are irreversibly damaged by the virus before delivery. Those infants would not benefit from postnatal therapy, but if infected fetuses could be detected before this irreversible stage has been reached, treatment in utero might have a significant effect on the course of the disease. Recent developments with effective antiviral agents may make such therapy possible (34). With the advances in antiviral chemotherapy, prenatal diagnosis of fetal infection could also lead to treatment of the mother with an anti-CMV agent known to cross the placenta. Ganciclovir is being administered to newborn infants congenitally infected with CMV, and encouraging results have been obtained (35). However, treatment of the affected neonate is probably too

late because much of the virus-induced damage to the infants may already have occurred before delivery.

For all these reasons, prenatal diagnosis of CMV infection of the fetus should be offered to primarily infected mothers. If this is not possible, the entire diagnosis of CMV infection during pregnancy is meaningless.

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REFERENCES

1. **Alford, C. A., S. Stagno, R. F. Pass, and W. J. Britt.** 1990. Congenital and perinatal cytomegalovirus infections. Rev. Infect. Dis. **12:**S745–S743.
2. **Bovicelli, L., L. F. Orsini, P. A. Grannum, M. C. Pittalis, C. Toffoli, and B. Dolcini.** 1989. A new funipuncture technique: two-needle ultrasound- and needle biopsy-guided procedure. Obstet. Gynecol. **73:**428–431.
3. **Britt, W. J., and C. A. Alford.** 1996. Cytomegalovirus. *In* B. N. Fields, D. M. Knipe, P. M. Howley, et al. (ed.), Fields virology, 3rd ed., vol. 2. Lippincott-Raven Publishers, Philadelphia, Pa.
4. **Catanzarite, V., and W. M. Dankner.** 1993. Prenatal diagnosis of congenital cytomegalovirus infection: false-negative amniocentesis at 20 weeks' gestation. Prenat. Diagn. **13:**1021–1025.
5. **Davis, L. E., G. V. Tweed, T. D. Y. Chin, and G. L. Miller.** 1971. Intrauterine diagnosis of cytomegalovirus infection: viral recovery from amniocentesis fluid. Am. J. Obstet. Gynecol. **109:**1217–1219.
6. **Demmler, G. J., G. J. Buffone, C. M. Schimbor, and R. A. May.** 1988. Detection of cytomegalovirus in urine from newborns by using polymerase chain reaction DNA amplification. J. Infect. Dis. **158:**1177–1184.
7. **Donner, C., C. Liesnard, F. Brancart, and F. Rodesch.** 1994. Accuracy of amniotic fluid testing before 21 weeks' gestation in prenatal diagnosis of congenital cytomegalovirus infection. Prenat. Diagn. **14:**1055–1059.
8. **Fowler, K. B., S. Stagno, and R. F. Pass.** 1997. Congenital cytomegalovirus (CMV) infection risk in future pregnancies & maternal CMV immunity, abstr. 191. *In* 6th International Cytomegalovirus Workshop.
9. **Freij, B. J., and J. L. Sever.** 1988. Herpesvirus infections in pregnancy: risk to embryo, and neonate. Clin. Perinatol. **15:**203–231.
10. **Ghidini, A., W. Sepulveda, C. J. Lockwood, and R. Romero.** 1993. Complications of fetal blood sampling. Am. J. Obstet. Gynecol. **168:**1339–1344.
11. **Gleaves, C. A., T. F. Smith, E. A. Shuster, and G. R. Pearson.** 1984. Rapid detection of cytomegalovirus in MRC-5 cells inoculated with urine specimens by using low-speed centrifugation and monoclonal antibody to an early antigen. J. Clin. Microbiol. **19:**917–919.
12. **Grose, C., and C. P. Weiner.** 1990. Prenatal diagnosis of congenital cytomegalovirus infection by virus isolation from amniotic fluid. Am. J. Obstet. Gynecol. **163:**1253–1255.
13. **Grose, C., T. Meehan, and C. P. Weiner.** 1992. Prenatal diagnosis of congenital cytomegalovirus infection by virus isolation after amniocentesis. J. Pediatr. Infect. Dis. **11:**605–607.
14. **Hogge, W. A., G. J. Buffone, and J. S. Hogge.** 1993. Prenatal diagnosis of cytomegalovirus (CMV) infection: a preliminary report. Prenat. Diagn. **13:**131–136.
15. **Hohfeld, P., Y. Vial, C. Maillars-Brignon, B. Vaudaux, and C. L. Fawer.** 1991. Cytomegalovirus fetal infection: prenatal diagnosis. Obstet. Gynecol. **78:**615–618.
16. **Kraat, Y. J., F. S. Stals, M. H. Christiaans, T. Lazzarotto, M. P. Landini, and C. A. Bruggeman.** 1996. IgM antibody detection of ppUL80a and ppUL32 by immunoblotting: an early parameter for recurrent cytomegalovirus infection in renal transplant recipients. J. Med. Virol. **48:**289–294.
17. **Lamy, M. E., K. N. Mulongo, J. F. Gadisseux, G. Lyon, V. Gaudy, and M. Van Lierde.** 1992. Prenatal diagnosis of fetal cytomegalovirus infection. Am. J. Obstet. Gynecol. **166:**91–94.
18. **Landini, M. P., and A. Ripalti.** 1982. A DNA nicking activity associated with the nucleocapsid of human cytomegalovirus. Arch. Virol. **73:**351–356.
19. **Landini, M. P., E. Rossier, and H. Schmitz.** 1988. Antibodies to human cytomegalovirus structural polypeptides during primary infection. J. Virol. Methods **22:**309–317.
20. **Landini, M. P., T. Lazzarotto, A. Ripalti, M. X. Guan, and M. La Placa.** 1989. Antibody response to recombinant lambda gt11 fusion proteins in cytomegalovirus infection. J. Clin. Microbiol. **27:**2324–2327.
21. **Lazzarotto, T., G. T. Maine, P. Dal Monte, A. Ripalti, and M. P. Landini.** 1997. A novel Western blot test containing both viral and recombinant proteins for anticytomegalovirus immunoglobulin M detection. J. Clin. Microbiol. **35:**393–397.
22. **Lazzarotto, T., P. Spezzacatena, P. Pradelli, D. A. Abate, S. Varani, and M. P. Landini.** 1997. Avidity of immunoglobulin G directed against human cytomegalovirus during primary and secondary infections in immunocompetent and immunocompromised subjects. Clin. Diagn. Lab. Immunol. **4:**469–473.
23. **Lazzarotto, T., A. Ripalti, G. Bergamini, M. C. Battista, F. Campanini, P. Spezzacatena, P. Pradelli, S. Varani, L. Gabrielli, G. T. Maine, and M. P. Landini.** 1998. Development of a new cytomegalovirus (CMV) immunoglobulin M (IgM) immunoblot for the detection of CMV-specific IgM. J. Clin. Microbiol. **11:**3337–3341.
24. **Lipitz, S., S. Yagel, E. Shalev, R. Achiron, S. Mashiach, and E. Schiff.** 1997. Prenatal diagnosis of fetal primary cytomegalovirus infection. Obstet. Gynecol. **89:**763–767.
25. **Lynch, L., F. Daffos, D. Emanuel, Y. Giovangrandi, R. Meisel, F. Forestier, G. Cathomas, and R. L. Berkowitz.** 1991. Prenatal diagnosis of fetal cytomegalovirus infection. Am. J. Obstet. Gynecol. **165:**714–718.
26. **Nelson, C. T., and G. J. Demmler.** 1997. Cytomegalovirus infection in the pregnant mother, fetus, and newborn infant. Infect. Perinatol. **24:**151–160.
27. **Nicolini, U., A. Kustermann, B. Tassis, R. Fogliani, A. Galimberti, E. Percivalle, M. G. Revello, and G. Gerna.** 1994. Prenatal diagnosis of congenital human cytomegalovirus infection. Prenat. Diagn. **14:**903–906.
28. **Revello, M. G., M. Zavattoni, E. Percivalle, P. Grossi, and G. Gerna.** 1989. Correlation between immunofluorescent detection of human cytomegalovirus immediate early antigens in polymorphonuclear leukocytes and viremia. J. Infect. Dis. **160:**159–160.
29. **Revello, M. G., F. Baldanti, M. Furione, A. Sarasini, E. Percivalle, M. Zavattoni, and G. Gerna.** 1995. Polymerase chain reaction for prenatal diagnosis of congenital human cytomegalovirus infection. J. Med. Virol. **47:**462–466.
30. **Stagno, S., and R. J. Whitley.** 1985. Herpesvirus infections of pregnancy. Part I. Cytomegalovirus and Epstein-Barr virus infections. N. Engl. J. Med. **313:**1270–1274.
31. **Stagno, S., R. F. Pass, C. Gretchen, W. J. Britt, R. E. Henderson, P. D. Walton, D. A. Veren, F. Page, and C. A. Alford.** 1986. Primary cytomegalovirus infection in pregnancy. Incidence, transmission to fetus, and clinical outcome. JAMA **256:**1904–1908.
32. **van der Biji, W. J., R. Torensma, W. J. Son, J. Schirm, A. M. Tegzess, and T. H. The.** 1988. Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leukocytes. J. Med. Virol. **25:**179–188.
33. **Weber, B., M. Opp, H. J. Born, U. Langenbeck, and H. W. Doerr.** 1992. Laboratory diagnosis of congenital human cytomegalovirus infection using polymerase chain reaction and shell vial culture. Infection **20:**155–157.
34. **Whitley, R. J., and D. W. Kimberlin.** 1997. Treatment of viral infections during pregnancy and the neonatal period. Clin. Perinatol. **24:**267–283.
35. **Whitley, R. J., G. Cloud, W. Gruber, G. A. Storch, G. J. Demmler, R. F. Jacobs, W. Dankner, S. A. Spector, S. Starr, R. F. Pass, S. Stagno, W. J. Britt, C. Alford Jr., S. Soong, X. J. Zhou, L. Sherrill, J. M. FitzGerald, and J. P. Sommadossi.** 1997. Ganciclovir treatment of symptomatic congenital cytomegalovirus infection: results of a phase II study. National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. J. Infect. Dis. **175:**1080–1086.