Anticytomegalovirus (Anti-CMV) Immunoglobulin G Avidity in Identification of Pregnant Women at Risk of Transmitting Congenital CMV Infection

TIZIANA LAZZAROTTO,1 PATRIZIA SPEZZACATENA,1 STEFANIA VARANI,1 LILIANA GABRIELLI,1 PAOLA PRADELLI,1 BRUNELLA GUERRA,2 AND MARIA PAOLA LANDINI1,*

Department of Clinical and Experimental Medicine, Section of Microbiology1 and Second Department of Obstetrics and Gynecology,2 Medical School, University of Bologna, Bologna, Italy

Received 6 July 1998/Returned for modification 17 August 1998/Accepted 30 September 1998

In this work, we show that the determination of the anticytomegalovirus antibody avidity carried out before week 18 of gestation is a helpful tool to identify women for enrollment in prenatal diagnosis. This procedure can identify all pregnant women who will give birth to an infected newborn.

Congenital cytomegalovirus (CMV) infection is the leading cause of congenital viral infection in developed countries, occurring in approximately 1% of all live births (1, 3, 4, 9, 18). Transmission of CMV to the fetus follows approximately 40% of primary maternal infections, whereas approximately 0.5% of women who are seropositive before pregnancy deliver infected infants (17). In addition, symptomatic infections and debilitat- ing sequelae are rare in congenitally infected children born to women with preexisting immunity to CMV (5).

Diagnosis of primary CMV infection in immunocompetent adults is accomplished by serological methods (3). CMV-specific immunoglobulin M (IgM) is a sensitive indicator of an ongoing or recent infection. However, it is not a specific indicator of primary infection, as it is often produced during non-primary infections (2, 10, 15). Another serological procedure useful in identifying primary infections is the determination of IgG avidity (6, 8, 11, 13).

In this work, we determined the avidity index (AI) of anti-CMV antibody in 76 pregnant women at risk of transmitting CMV to their offspring as well as in 20 pregnant women at no risk of transmitting CMV. All the women went through prenatal diagnoses, and pregnancy outcomes were monitored.

Between January 1994 and May 1998, 76 pregnant women were enrolled in the prenatal diagnosis program for CMV infection either because of a seroconversion for CMV during the first trimester of pregnancy (n = 15) or because of the presence of CMV-specific IgM (n = 61).

Twenty pregnant women at no risk of fetal CMV transmission (as determined by a negative IgM test) but who had amniotic fluid (AF) and fetal blood taken for fetal karyotype assessment constituted a control group. Twenty milliliters of AF was collected by amniocentesis at 21 to 23 weeks' gestation with informed consent from all women. CMV DNA was individually extracted from 3 to 6 aliquots of AF (100 µl each), and PCR was carried out as described in detail previously (12). AF was considered positive if at least one of the aliquots was positive.

Congenital CMV infection in a newborn was determined by CMV isolation (7) from urine or saliva during the first week of life. The determination of IgG avidity was carried out using a commercial kit (Cytomegalovirus IgG avidity EIA WELL; RADIM, Rome, Italy).

As shown in Table 1, we obtained blood samples from 76 pregnant women during the early phase of pregnancy (6 to 18 weeks' gestation; mean, 13.6 weeks). Anti-CMV antibody avidity was determined, and in only six cases it was not possible because of an IgG level that was too low. Of these women, one with congenital hypogammaglobulinemia who was found to be PCR positive in AF testing decided to interrupt her pregnancy. No sign of CMV infection was found in the fetus. The other five women continued their pregnancies, and no congenitally infected newborn was documented. In the remaining 70 samples, the AI was determined, and the women were classified into one of the following three groups on the basis of the result obtained: low AI (38 cases), moderate AI (6 cases), and high AI (26 cases). Of the 38 women who showed a low avidity, 17 had the viral genome in the AF (45%) as detected by PCR. Thirteen women (seven who seroconverted and six who were IgM positive) continued their pregnancies, and the virus was isolated from six newborns (four asymptomatic and two symptomatic). Four women (one who seroconverted and three who were IgM positive) decided to interrupt their pregnancies, and all four fetuses showed the presence of CMV in multiple organs (data not shown). A detailed analysis of the results obtained on prenatal diagnoses has been recently published (12).

Among the six women with moderate avidity, two had the viral genome in the AF (33%). All of them decided to continue their pregnancies, and no congenital infection was documented in their newborns. Finally, of 26 women with high AI, 3 had the viral genome in the AF (11%). All of them continued their pregnancies and no congenital infection was documented in their newborns.

Later during pregnancy (at the time of amniocentesis, i.e., after 21 to 23 weeks' gestation), another blood sample was obtained from the same 76 pregnant women, and the AI was...
Control population of 76 women, and in only 10 of the samples did we document a moderate or high avidity.

Pregnancy, 60% of the women who will transmit the infection more, if the determination of AI is carried out later on during pregnancy (before 18 weeks), it can identify all pregnant women who will develop a congenital infection in fetuses or newborns. This is probably due to the high sensitivity of the procedure, which detects a viral load so low as to be cleared by the fetal defenses, and is consistent with other published data (14, 16). Quantitative PCR is in progress to verify this hypothesis.

In conclusion, the early determination of anti-CMV antibody avidity is a helpful tool to identify a subgroup of IgM-positive women to enroll in prenatal diagnosis.

This work was partially supported by Istituto Superiore di Sanità, Progetto, Sicurezza del Sangue e dei suoi Prodotti (1996) and the AIDS projects (1997 and 1998) and by the Italian Ministry of Education (40 and 60%).

### REFERENCES

1. Alford, C. A., S. Stagno, R. F. Pass, and W. J. Britt. 1990. Congenital and perinatal cytomegalovirus infections. Rev. Infect. Dis. 12:S743–S745.
2. Basson, J., J. C. Tardy, and M. Aymard. 1989. Pattern of anti-cytomegalovirus IgM antibodies determined by immunoblotting. A study of kidney graft recipients developing a primary or recurrent CMV infection. Arch. Virol. 108:259–270.
3. Britt, W. J., and C. A. Alford. 1996. Cytomegalovirus, p. 2493–2523. In B. N. Fields, D. M. Knipe, P. M. Howley, et al. (ed.), Fields Virology, 3rd ed. Lippincott-Raven Publishers, Philadelphia, Pa.
4. 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.
5. Fowler, K. B., S. Stagno, R. F. Pass, W. J. Britt, T. J. Boll, and C. A. Alford. 1992. The outcome of congenital cytomegalovirus in relation to maternal antibody status. N. Engl. J. Med. 326:663–667.
6. Genevieve, R. E., P. Barjot, M. Campet, A. Vabret, M. Herlicoviez, G. Muller, G. Levy, B. Guillot, and F. Freymuth. 1996. Evaluation of virological procedures to detect fetal human cytomegalovirus infection: avidity of IgG antibodies, virus detection in amniotic fluid and maternal serum. J. Med. Virol. 50:9–15.
7. 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.
8. Grangeot-Keros, L., M. J. Mayaux, P. Lebon, F. Freymuth, G. Eugene, R. Stricker, and E. Dussaix. 1997. Value of cytomegalovirus (CMV) IgG avidity for the diagnosis of primary CMV infection in pregnant women. J. Infect. Dis. 175:944–946.

### TABLE 1. Avidity index of anti-CMV IgG at two different gestation times in relation to prenatal diagnosis carried out by PCR in amniotic fluid and pregnancy outcomes in pregnant women at risk of transmitting a CMV infection and in a control population

| Classification of AI of: | n | No. of PCR-positive prenatal diagnoses of amniotic fluid | No. of infected fetuses or newborns | SNS (%) | SPE (%) | PPV (%) | NPV (%) |
|--------------------------|---|-------------------------------------------------------|------------------------------------|---------|---------|---------|---------|
| **Women at risk**         |   |                                                       |                                     |         |         |         |         |
| After 6–18 weeks of gestation | |                                                       |                                     |         |         |         |         |
| Low                      | 38 | 17                                                   | 10                                 | 100     | 57.5    | 26.3    | 100     |
| Moderate                  | 6  | 2                                                    | 0                                  |         |         |         |         |
| High                      | 26 | 3                                                    | 0                                  |         |         |         |         |
| Not determinable*         | 6  | 1                                                    | 0                                  |         |         |         |         |
| At time of amniocentesis (21–23 weeks) | |                                                       |                                     |         |         |         |         |
| Low                      | 32 | 12                                                   | 6                                  | 60      | 60.6    | 18.8    | 90.9    |
| Moderate                  | 6  | 3                                                    | 1                                  |         |         |         |         |
| High                      | 32 | 7                                                    | 3                                  |         |         |         |         |
| Not determinable*         | 6  | 1                                                    | 0                                  |         |         |         |         |
| **Control population**    |   |                                                       |                                     |         |         |         |         |
| High (IgM negative)       | 20 | 0                                                    | 0                                  |         |         |         |         |

*a SNS, sensitivity (true positives/total infected fetuses or newborns × 100).

*b SPE, specificity (true negatives/total uninfected fetuses or newborns × 100).

*c PPV, positive predictive value (true positives/true positives + false positives × 100).

*d NPV, negative predictive value (true negatives/true negatives + false negatives × 100).

*Not determinable because of anti-CMV IgG levels too low to determine AI.
9. Ho, M. 1990. Epidemiology of cytomegalovirus infections. Rev. Infect. Dis. 12:S701–S710.
10. 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.
11. 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.
12. Lazzarotto, T., B. Guerra, P. Spezzacatena, S. Varani, L. Gabrielli, P. Pradelli, F. Rumpianesi, C. Banzi, L. Bovicelli, and M. P. Landini. 1998. Prenatal diagnosis of congenital cytomegalovirus infection. J. Clin. Microbiol. 36:3540–3544.
13. Lutz, E., K. N. Ward, and J. J. Gray. 1994. Maturation of antibody avidity after primary human cytomegalovirus infection is delayed in immunosuppressed solid organ transplant patients. J. Med. Virol. 44:317–322.
14. 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.
15. Nielsen, C. M., K. Hansen, H. M. K. Andersen, J. Gerstoft, and B. F. Vestergaard. 1987. An enzyme labelled nuclear antigen immun assay for detection of cytomegalovirus IgM antibodies in human serum: specific and nonspecific reactions. J. Med. Virol. 22:67–76.
16. 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.
17. Stagno, S., R. F. Pass, M. E. Dworsky, R. E. Henderson, E. G. Moore, P. D. Walton, and C. A. Alford. 1982. Congenital cytomegalovirus infection: the relative importance of primary and recurrent maternal infection. N. Engl. J. Med. 306:945–949.
18. Whitley, R. J., and D. W. Kimberlin. 1997. Treatment of viral infections during pregnancy and the neonatal period. Clin. Perinatol. 24:267–283.