Dot Hybridization and Polymerase Amplification of DNA in the Prenatal Diagnosis of Cytomegalovirus Infection

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Molecular biology techniques (dot hybridization and polymerase amplification of DNA) are used to diagnose cytomegalovirus infection in pregnant women and newborns. It is concluded from the results that dot hybridization is a convenient tool in screening studies and that polymerase DNA amplification followed by verification of the reaction product using restriction enzymes and blot hybridization makes the diagnosis of this disease more reliable and may find wide application in practical laboratories.

Key Words: *cytomegalovirus infection; prenatal diagnosis; dot hybridization; polymerase DNA amplification*

Cytomegalovirus (CMV) infection occupies a special position among intrauterine infections because of its wide distribution, its not infrequently latent chronic course, and the absence of effective preventive or therapeutic measures, and also because the manifestations of intrauterine infection are delayed. In 1974, CMV infection was included in a syndrome of intrauterine infectious pathology, the so-called TORCH syndrome, which also includes toxoplasmosis, rubella, and herpes [7].

Although CMV infection is widely distributed and has grave consequences for the fetus, its clinical manifestations in pregnant women tend to be scarce. In most cases, CMV infection is either symptomless or manifests itself as an acute respiratory disorder with few symptoms or as a mononucleosis syndrome. CMV infection acquired during pregnancy may result in spontaneous abortion, premature labor, or hydramnions [4].

The incidence of congenital CMV infection among live-born infants ranges from 0.5 to 2%. About 5-10% of congenitally infected newborns have clinical manifestations of the disease such as severe neurological disorders, a hemorrhagic syndrome, jaundice, and splenomegaly [6,11]. A frequently encountered feature of congenital CMV infection is the delayed onset of central nervous system abnormalities, which are manifested as mental retardation by the age of 5-6 years in 20-30% of children infected *in utero* [9].

The quest for specific and highly sensitive methods of diagnosis has led to the use of molecular biology techniques such as dot hybridization and the polymerase chain reaction for DNA synthesis in addition to cytological, virological, and serological (e.g. enzyme-linked immunosorbent assay) methods widely employed for diagnosing CMV infection.

First described by Saiki in 1985 [10], the technique of polymerase DNA amplification has found wide application in biology and medicine for the identification of particular genomic sequences. In the Russian-language literature, there are only a few publications concerning the use of molecular biology methods to detect CMV, and the methodological aspects of such detection have been covered quite inadequately.

MATERIALS AND METHODS

Clinical specimens from patients suspected of having CMV infection were analyzed. For cytological

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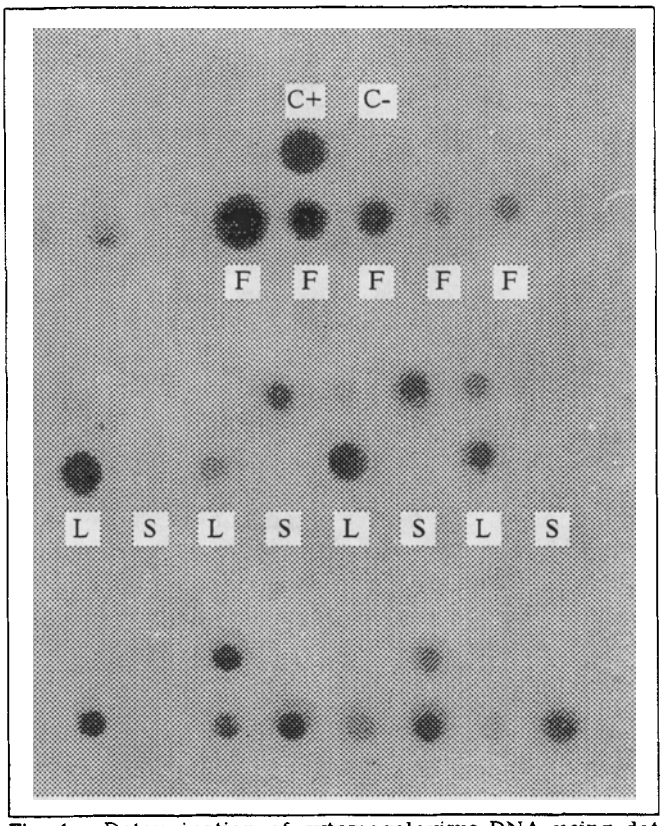

Fig. 1. Determination of cytomegalovirus DNA using dot hybridization. C^+ = positive control; C^- = negative control; F = organs of fetuses that died antenatally; L = lymphocytes; S = serum. The autoradiograph was recorded for 72 h.

assays, urine and saliva smears were stained by Pappenheim's method and examined under a microscope (×40). Cytomegalic cells had a clear halo around the nucleus, resulting in an "owl's eye" appearance.

In serological studies, specific IgG and IgM antibodies were detected in sera from pregnant women and newborns by enzyme-linked immunosorbent assay using commercial kits produced by Abbott and DIA-Plus.

DNA sequences of CMV were determined in blood, lymphocytes, urine sediments, placental and fetal tissues, chorion biopsy specimens, organs of dead fetuses, and also in cultures of pulmonary fibroblasts from human embryos infected with CMV strain AD169 (positive controls). DNA was isolated from the clinical specimens by treatment with proteinase K for 3 h at 56°C followed by purification with phenol-chloroform and sedimentation with two volumes of ethanol [2].

Dot hybridization : the DNA under study was applied to a capron filter and hybridized with a γ-^{32}P-ATP-labeled oligonucleotide probe using T4 polynucleotide kinase. Autoradiographs were obtained for 7 days at -20°C.

For the *polymerase chain reaction,* two pairs of primers from exons 1 and 4 of the MIE region of CMV were used, which enable fragments having 147/5, 3/ and 240/12, 13/base pairs to be synthesized, with the following nucleotide compositions:

Pair 1: 5'AGCTGCATGATGTGAGCAAG3'
5'GAAGGCTGAGTTCTTGGTAA3'

Pair 2: 5'CCCGACTTTACCATCCAGTA3'
5'AAGACGAAGAGGAACTATCT3'.

The reaction was carried out in Eppendorf microcentrifuge tubes containing a mixture of the following composition: 16.6 mM ammonium sulfate; 67 mM Tris-HCl, pH 8.8; 6.7 mM magnesium chloride; 10 mM β-mercaptoethanol; 6.7 μM EDTA; 170 μg/ml bovine serum albumin, fraction 5 (Serva); 200 μM of each of the deoxytriphosphates (Vektor Enterprise, Novosibirsk, Russia); 2 units of thermostable DNA polymerase from *Thermus thermophilus* (BioFarmToks Enterprise, St. Petersburg, Russia); 1 ng of each primer; and 1 ng of the test DNA. The reaction products were visualized by electrophoresis in 8% polyacrylamide gel followed by staining with ethidium bromide. Specificity of these products was evaluated by means of blot hybridization and restriction enzyme digest analysis. The amplification product having 147 base pairs was cleaved by Pst I restriction enzyme into two fragments of 50 and 97 base pairs, while the amplification product with 240 base pairs was cleaved by Hga I restriction enzyme into two fragments of 101 and 139 base pairs.

As the positive control, the pO plasmid constructed by Oram [8] and kindly donated by Professor Southern (Oxford) was used. The plasmid vector was constructed on the basis of pAT 153 vector and contained the MIE fragment of CMV.

RESULTS

More than three years' experience with the use of enzyme-linked immunoassays has led us to conclude that the efficacy of this method in recognizing CMV infection is low because of the high prevalence of seropositive individuals among pregnant women. Moreover, the level of IgM antibodies does not always correlate with the time elapsed after the onset of the disease. Another important consideration is the high cost of the kits, which severely limits the use of this method for screening purposes under our conditions.

The cytological method of diagnosing CMV infection has proved to be simple and readily accessible, but its low sensitivity makes it necessary to employ additional diagnostic tests.

The results of dot hybridization we obtained confirmed, in all cases, the presence of CMV se-

quences in nucleated cells of maternal blood during the acute phase of the infection. We traced the process of virus transfer from mother to fetus across the placenta. Viral DNA was also identified in fetal tissues of a woman with habitual abortions (Fig. 1).

Dot hybridization has shown itself as a sensitive and convenient tool for examining a large number of specimens simultaneously. Nevertheless, in view of the not very high detection limits of this method, it is advisable to use polymerase DNA amplification for the demonstration of virus sequences.

The polymerase chain reaction yielded amplification products of 147 and 240 base pairs, and the specificity of these products was verified using blot hybridization and restriction enzyme digest analysis (Fig. 2). In this way, the diagnosis of CMV infection was confirmed in 20 pregnant women, 4 newborns, and in 2 fetuses that died antenatally.

Our results have led us to the conclusion that the polymerase chain reaction is an effective means of diagnosing intrauterine CMV infection, for this reaction is highly sensitive, enables a wide diversity of clinical specimens to be analyzed (ranging from biological fluids to various organs and tissues), yields results rapidly enough (within 48 h), and obviates the need to employ a radioactive label.

Because no effective therapy is available for CMV infection, its early recognition is one of the most important measures for preventing the birth of infants with severe psychosomatic disorders. With molecular biology techniques, it is possible to reliably confirm the presence of CMV in pregnant women, fetuses, and neonates at risk of developing grave disease. The method of dot hybridization has proved convenient for screening studies. The polymerase chain reaction followed by verification of the reaction product using restriction enzymes and blot hybridization makes the diagnosis of CMV infection more reliable and can be expected to find wide use in practical laboratories in the near future.

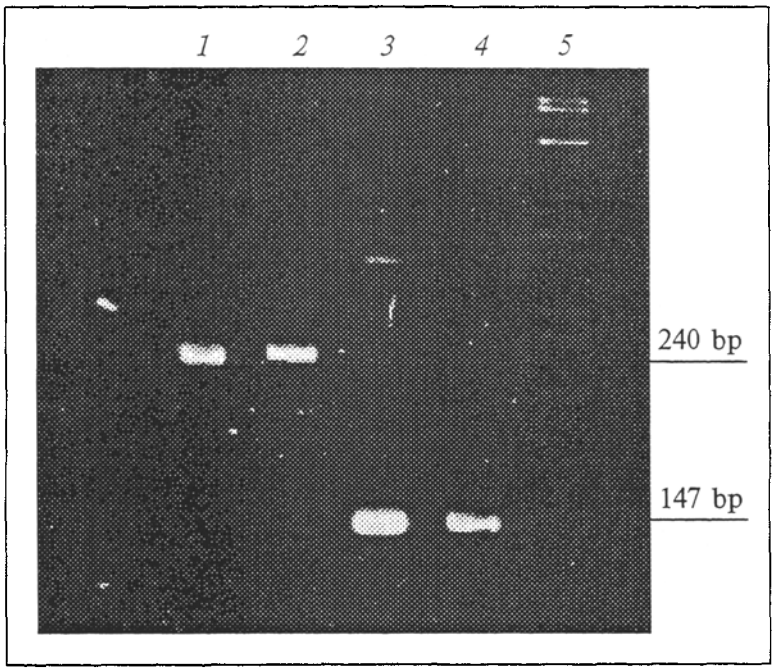

Fig. 2. Determination of cytomegalovirus DNA using polymerase DNA amplification: *1* and *3*) amplification products of plasmid DNA, having 240 and 147 base pairs, respectively; *2* and *4*) amplification products of DNA isolated from the brain of a fetus that died antenatally; the products have 240 and 147 base pairs, respectively. Electrophoresis in 8% polyacrylamide gel followed by staining with ethidium bromide and photography in ultraviolet light.

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