Chapter 18 Diagnosis of Prenatal Rubella by Polymerase Chain Reaction

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Summary

First trimester rubella carries a high risk of congenital abnormalities in the fetus. Evidence of an intrauterine infection is therefore of value for prenatal counselling. We present data to show that the polymerase chain reaction has advantages over other conventional techniques in confirming such a diagnosis, but problems remain in its application, and these are also discussed.

Introduction

Maternal viral infection during pregnancy is known frequently to involve the fetus. The incidence of fetal infection and the risk of congenital deformities are particularly high with rubella (Miller et al. 1982). When fetal infection is suspected, a direct intrauterine diagnosis can provide invaluable information for prenatal counselling. This is now possible because of the availability of fetal specimens which can be obtained with relatively low risk in suitably equipped and staffed obstetrics centres.

Several approaches have been adopted to diagnose fetal rubella infection. One involves the detection of specific immunoglobulin (IgM) in fetal blood by standard immunoassays (Daffos et al. 1984; Enders 1987; Ho-Terry et al. 1988). However, since a fetus does not usually develop IgM antibodies at a detectable level until the 18th–20th weeks of gestation, this method of diagnosis is not satisfactory in the care of women who are infected early in pregnancy when the risk of fetal deformities is highest (Miller et al. 1982). Viral infections can affect the development of the immunological system in utero and transplacental maternal IgG antibodies are also liable to modulate the fetal immunological response. False-negative results are sometimes obtained when fetal rubella is diagnosed in this way (Daffos et al. 1984; Enders 1987).

As an alternative, fetal rubella infection can be diagnosed by the detection of infectious virus in products of conception, e.g., chorionic villus samples.
(CVS) and amniotic fluid during pregnancy or placental and fetal tissues after therapeutic abortion. This method of diagnosis has been carried out successfully in a limited number of laboratories, but in general the rate of rubella virus isolation after first trimester infection is variable (68%–90%) and dependent on viral load (Cooper et al. 1968; Rawls et al. 1968). A missed diagnosis of first trimester rubella presents a serious risk of delivery of an infant with congenital rubella defects.

We have examined aborted specimens from women with first trimester rubella by virus isolation. These specimens were cocultivated with VERO, African green monkey kidney cells for 4–5 days under standard laboratory conditions (Herrmann 1979). Any rubella virus, if present, is “biologically amplified”. Virus released into the inoculated medium was detected in RK13, rabbit kidney cells by histochemical staining of infected cells with a mixture of rubella monoclonal antibodies (Turner 1986). In addition, RNA from the same culture was analysed by Northern blotting using a 32P-labelled cloned rubella cDNA probe (Terry et al. 1986).

Results obtained from this study identified two categories of specimens with evidence of rubella virus infection. Neither of these categories show organ specificity. Category 1 (19/40) represents positive isolates which yield infectious rubella virus and rubella-specific RNA (40S genomic RNA and/or 24S mRNA) in inoculated cells (Fig. 1a, lanes 1 and 5). Category 2 (21/40) represents specimens from which no infectious virus can be isolated but which contain rubella-specific subgenomic and sub-24S RNA species demonstrable by Northern blotting (Fig. 1a, lane 3). The precise mechanism of generation of subgenomic or sub-24S RNA species in rubella-infected human tissue is not known. However, virus particles with subgenomic RNA species frequently develop in rubella-infected tissue culture systems (Terry et al. 1986; Frey and Hemphill 1988). These defective interfering (DI) particles are not infectious but are known to interfere with the infectivity of intact particles.

To try to identify specimens which have been infected with rubella but which may contain very low levels of intact rubella RNA or which are not capable of being biologically amplified due to interference by DI particles, we have employed hybridisation of RNA extracted from specimens with a 32P-labelled, cloned, rubella cDNA probe. Reconstruction experiments show that 1–2 pg of rubella-specific RNA can be detected in this way. This represents about 1000 infected cells. Assuming that between 1/1000 and 1/250000 fetal cells are infected in intrauterine rubella infection (Rawls et al. 1968), this quantity of rubella RNA would require a specimen containing $10^6$ to $2.5 \times 10^8$ fetal cells, equivalent to a wet weight of 3–750 mg of tissue. Although this poses no problem for retrospective studies using aborted specimens, it is a serious limitation for prenatal diagnosis since the average wet weight of a CVS is about 20–30 mg, so that a positive result will be obtained only in specimens with a relatively high rate of rubella infection, i.e., at least 1 in 10 000 fetal cells. About 30% of CVS made available for our study fall short of 20 mg wet weight. It is therefore evident that diagnosis of intrauterine rubella infection by detection of specific RNA would be more efficient and reliable if viral se-