Chlamydia trachomatis detected in human placenta

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Abstract

Aims—To evaluate the relation between *Chlamydia trachomatis* infection and stillbirth, placental tissue was studied for the presence of *C trachomatis*.

Methods—Paraffin wax embedded placental tissue of a stillbirth fetus, born at the 36th week of gestation to a 21 year old mother with high serum antibody titres to *C trachomatis* immunotypes during pregnancy and who was culture positive to *C trachomatis* three years previously, was studied by in situ hybridisation, polymerase chain reaction, and immunohistochemistry for the presence of *C trachomatis*.

Results—*C trachomatis* was detected in placental specimens by in situ hybridisation and alkaline phosphatase antialkaline phosphatase staining in several sections, whereas control tissues were uniformly negative, indicating the presence of *C trachomatis* nucleic acid and antigen in the placenta.

Conclusion—This is the first reported case in which *C trachomatis* has been demonstrated in the human placenta.

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Keywords: *Chlamydia trachomatis*; in-situ hybridisation; placenta

Genital chlamydial infection is recognised as the world's most common sexually transmitted disease.¹ In the majority of cases the condition is asymptomatic. *Chlamydia trachomatis* is associated with various complications of pregnancy² and appears to be associated with premature birth and neonatal problems.^{3,4} One case leading to fetal death has been reported although the route of infection was unknown.⁵ In this study, we established an in situ hybridisation technique and a modified alkaline phosphatase antialkaline phosphatase staining (APAAP) method for the detection of *C trachomatis*, which we used to demonstrate the presence of *C trachomatis* in placental specimens of a stillbirth fetus.

Methods

CASE HISTORY

A stillbirth fetus was born to a 21 year old mother at the 36th week of gestation. The placenta was abrupted and a male fetus was delivered by caesarean section. At necropsy the fetus appeared normal. By microscopic examination, petechiae were observed in the lungs, pericardium, and thymus and hyperaemia

appeared in the adrenal glands and spleen. The mother had high IgG antibody titres (1:128) to *C trachomatis* pooled immunotypes GFK and BED at the 13th week of gestation while IgM to *C trachomatis* was negative when evaluated in a microimmunofluorescence test. Using an antibody screen against 16 microbes, the mother had a high serum IgG antibody level to herpes simplex virus (HSV) type 2, other antibody levels were unremarkable. Culture from a cervical specimen had been positive to *C trachomatis* three years earlier. At that time the patient had been treated for 10 days with doxycycline. During the present pregnancy no cultures were performed.

IN SITU HYBRIDISATION

A digoxigenin labelled RNA probe was constructed from *C trachomatis* L₂ 7.5 kb plasmid (kindly donated by Dr S Larsen, Indiana University School of Medicine, Indiana, USA).⁶ Sections of paraffin embedded placental tissue, cut at 4 µm were floated on to silane treated slides. The tissue sections were deparaffinised and rehydrated. For in situ hybridisation, the tissue sections were fixed in 4% paraformaldehyde and digested with proteinase K. Tissue sections were immediately refixed in 4% paraformaldehyde and acetylated in triethanolamine (pH 8.0) containing acetic anhydride. After specimen dehydration through graded ethanols, they were prehybridised by adding hybridisation buffer (2× saline sodium citrate (SSC), 50% deionised formamide, 1× Denhardt's, 500 µg/ml yeast tRNA, 0.1% Tween 20, 0.1% CHAPS (Sigma, St Louis, Missouri, USA), 5 mM ethylenediamine tetraacetic acid (EDTA), and 100 µg/ml heparin (Sigma). For hybridisation, 60 µl of hybridisation mix containing 0.2 ng/µl of probe was placed on to the tissues. Coverslips were sealed with rubber cement, and the tissues were denatured at 80°C for 10 minutes and hybridised with the labelled probe at 37°C overnight. The post-hybridisation washes were performed under highly stringent conditions.

Controls included tissue sections with hybridisation solution without probe and tissue sections with hybridisation solution containing a non-specific probe prepared from the vector. To remove any unbound probe, sections were treated with RNase (100 µg/ml) at 37°C. Following post-hybridisation washes, the hybridisation signal was detected according to the instructions of the DIG Nucleic Acid detection Kit (Boehringer Mannheim, Mannheim, Germany). All tissue sections were tested blindly.

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Figure 1 In situ hybridisation of placental tissue and controls. (A) Hybridisation of chlamydial DNA in human placenta with the *Chlamydia trachomatis* plasmid DNA derived, dUTP labelled RNA probe. (B) No hybridisation in the same placental tissue with the vector (pGEM-3Zf(-)) DNA derived, dUTP labelled RNA probe (negative control). (C) Hybridisation of chlamydial DNA in McCoy cell culture with the *C trachomatis* probe. (D) No hybridisation with the vector probe in infected McCoy cells (negative control).

POLYMERASE CHAIN REACTION AMPLIFICATION

The oligonucleotide primers (KL1, 5'-TCCGGAGCGAGTTACGAAGA-3'; KL2, 5'-AATCAATGCCCCGGGATTGGT-3') were used to amplify *C trachomatis* plasmid DNA. We also used β globin primers PC03, PC04 (5'-ACACAACTGTGTTCCTAGC-3'; 5'-CAACTTCATCCACGTTTCACC-3') to monitor sample preparations for the presence of possible inhibitors. Sections of paraffin wax embedded placental tissue were cut at 4 μ m and placed into sterile tubes. The tissue sections were deparaffinised with xylene and digested with proteinase K (1 mg/ml) (Boehringer Mannheim) in lysis buffer (50 mM Tris HCl, pH 7.5, 1 mM EDTA, 1% Tween 20) at 37°C overnight. The reaction was stopped by heating to 95°C for eight minutes. After phenol and chloroform extraction the samples were precipitated with ethanol. Amplification was performed in 100 μ l reaction mixes for KL1-KL2 (200 μ M dNTPs (Pharmacia, Uppsala, Sweden), 1 μ M each primer, 2 U Taq polymerase, 10 $\times$ polymerase chain reaction (PCR) buffer (Perkin Elmer, Norwalk, Connecticut, USA)) and for PC03-PC04 (200 μ M dNTPs, 1 μ M each primer, 2 U Taq polymerase, 1.5 mM MgCl₂, 10 $\times$ PCR buffer without MgCl₂), each containing 10 μ l of the sample. The reaction mixtures were heated to 95°C for five minutes and then subjected to 35 cycles of 95°C for one minute, 55°C for one minute, and 72°C for two minutes; a final

extension was carried out at 72°C for 10 minutes. The amplification reaction mixture (10 μ l) was analysed by gel electrophoresis.⁷

IMMUNOHISTOCHEMICAL STAINING

APAAP staining of chlamydial inclusions in tissues was carried out using a modification of the procedure described by Mahony *et al.*⁸ A genus specific mouse monoclonal antibody recognising chlamydial lipopolysaccharide (Biokit, Barcelona, Spain) was used. To control for non-specific staining, duplicate tissue sections were incubated with mouse ascites fluid. The sections were examined by light microscopy.

OTHER TESTS

A routine microscopic histopathological study was performed using formalin fixed paraffin embedded tissue cut at 3 μ m and stained with haematoxylin and eosin. Microimmunofluorescence tests for the detection of the specific antibodies were performed using the antigens of *C trachomatis* immunotype pools CJH1, GFK, and BED (Washington Research Foundation, Seattle), *C pneumoniae* strain TWAR (Washington Research Foundation), and *C psittaci* OA and 6 BC (Slovak Academy of Sciences, Bratislava, Slovakia). Antibodies to 16 microbes were determined from maternal serum samples using enzyme immunoassay. PCR assays were performed in the paraffin embedded tissue sections using primers from the DNA polymerase gene for HSV-1, HSV-2,

Figure 2 Immunoenzymatic APAAP staining of placental tissue and controls. (A) Detection of chlamydial antigen in placental tissue using a genus specific monoclonal antibody. (B) No staining is seen in a parallel section from the same tissue stained with normal mouse ascitic fluid. (C) *Chlamydia trachomatis* inclusions in McCoy cell culture are detected by APAAP staining using a genus specific monoclonal antibody. (D) Negative control (uninfected McCoy cells).

Figure 3 Routine histological examination of placental tissue shows very little, non-specific degenerative changes such as knotting of the syncytiotrophoblasts, small intervillous thrombus, and patchy necrosis. There is no inflammatory reaction. (Haematoxylin and eosin, original magnification $\times 160$.)

and varicella zoster virus and detected with luminometric microplate hybridisation.⁹ For *Toxoplasma gondii* PCR, the primers from the BI gene were used. In all PCRs, the paraffin embedded tissue sections were deparaffinised with xylene and digested by proteinase K as described for *C trachomatis* PCR.

Results

C trachomatis DNA and antigen were detected by in situ hybridisation and APAAP staining in

placental tissue from the fetus. Criteria for positive hybridisation were adopted from studies with infected and uninfected McCoy cells (fig 1).

Cytoplasmic chlamydial inclusions were clearly identifiable with APAAP staining. The inclusions appeared bright red and granular against a background of blue nuclei (fig 2).

Routine histological examination revealed no specific signs of chlamydial infection (fig 3). In the PCR test for *C trachomatis*, the placental tissue remained negative while β globin gave a strong and specific band on gel electrophoresis indicating the reliability of the test. PCR tests for HSV-1, HSV-2, varicella zoster virus, and *Toxoplasma gondii* gave negative results for the placental tissue sections.

Discussion

To our knowledge this is the first case in which *C trachomatis* has been identified in the human placenta. Presence of the organism has been suspected but evidence has been either serological or hypothetical.^{10 11} The presence of *C trachomatis* in the placenta represents a potentially significant threat to the fetus. Infection of the placenta will directly expose the fetus to an infection against which a specific therapy is available. *C trachomatis* has been detected in fetal lung in a stillbirth case⁵ although the route of invasion was unknown. *C trachomatis* has also been detected in endometrium and tubal tissue.²

C. psittaci is known to cause abortions in sheep and it can be transmitted to humans.^{12 13} In the present case, because only paraffin embedded blocks were available, chlamydial culture from placental tissue was not attempted. The in situ hybridisation and APAAP studies provide evidence to suggest that nucleic acid and antigen were present. Tests for other microbes were consistently negative. The failure of PCR to detect *C. trachomatis* may be explained by the amount of inhibitory material present in tissues. By our PCR test, β globin was identified, but its concentration is higher than that of *C. trachomatis* nucleic acid. Using the same technique we have been able to identify the nucleic acid from other specimens.

Routine histological staining showed only non-specific degenerative changes. These changes were not suggestive of an ongoing infection.

C. trachomatis grows well in trophoblasts in vitro,¹⁴ as well as in amniotic cells.¹⁵ Chlamydial infections may cause necrosis as does *C. psittaci* in animal tissues.¹⁶ Thus, the presence of *C. trachomatis* in the placenta seems conceivable and, consequently, raises important questions regarding complications in pregnancy. Because *C. trachomatis* has been recognised as an increasingly prevalent and important genital infection, recommendations for screening have been reported, and it has been included in the antenatal care guidelines, at least in Germany.¹⁷

The complications of pregnancy associated with chlamydial infections have often been attributed to induction of heat shock protein, hypersensitivity, a lymphoproliferative reaction, or an autoimmune process.¹⁸ However, the presence of *C. trachomatis* in the placenta alters the potential impact of the infection as a cause of fetal complications and the consequent value of antimicrobial agents in such cases. In a large controlled trial, a seven day course of doxycycline and a single, 1 g dose of azithromycin were equally effective.¹⁹ During pregnancy, azithromycin is a valid treatment option for patients who cannot tolerate erythromycin²⁰ and it has been raised to the first line therapies of infections caused by *C. trachomatis*. There are reports of improved pregnancy outcome after successful antibiotic therapy in cases where *C. trachomatis* has been identified in cervical specimens.²¹

C. trachomatis is one of the major causes of infections affecting mankind; several hundred millions of individuals are infected worldwide and, consequently, the number of fetuses exposed to chlamydial infection and associated complications may be high. The infection, once acquired, tends to persist and is usually symptom free.²² Awareness of *C. trachomatis* infections may improve both the diagnostics, by screening for *C. trachomatis* asymptomatic infections, and the therapy of these infections,

and result in a better outcome for exposed children.

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