Chlamydia trachomatis has recently been recognised as one of the most prevalent ocuogenital pathogens. Some workers showed that C trachomatis can be transmitted to infants from the mother.1-2 The risk of inclusion conjunctivitis in infants born to mothers with cervical infections ranges from 18 to 50%.3 Moreover, nasopharyngeal infection may result in pneumonia,4 and the risk of transmission from infected mothers to infants is 3–18%.

Although several seroepidemiological studies of children from infancy to puberty have shown a high incidence of chlamydial infection,5-7 exactly how C trachomatis is transmitted remains to be determined. The role of C trachomatis infection in spontaneous abortion and premature birth is also unclear. Recently, however, the correlation between defects in pulmonary function in pneumonia and infection with C trachomatis in newborn babies has been reported.8

In the present study serum IgM antibodies against C trachomatis were determined in premature infants with chronic respiratory disease and sections of lung tissue collected at necropsy and biopsy were also tested for the presence of C trachomatis antigen.

Material and methods

Case reports

Case I The mother of this girl was 21 years old, a gravida II para I without a history of urethritis or salpingitis. At 19 she had had a normal pregnancy and delivery. The second pregnancy was normal until the 27th week of gestation, when the mother was admitted to hospital for impending labour and deceleration of fetal heart sound. Although the fetus was a breech presentation, the delivery was normal. The fetal membrane and placenta were normal, but the amniotic fluid was not clear. After one minute the Apgar score was 1. The birth weight was 980 g.
During the first hours of life the infant had systemic cyanosis and hypothermia and her muscular tonus was hypotonic. She was intubated and given intermittent mechanical ventilation with a supplement of oxygen. Ampicillin was started intravenously but discontinued when the results of a test of C reactive protein were negative. She developed progressive respiratory difficulties. A roentgenogram showed bilateral parenchymal changes. Some hours later the oxygen pressure decreased. Treatment was continued with positive end expiratory pressure (fractional inspired oxygen 0:7) and theophylline. She also received 12 ml fresh whole blood. At 36 hours of age a chest radiograph showed bilateral reticulonodular changes (Fig. 1). Laboratory data included the following: white cell count 9·8 × 10⁹/l with 42% neutrophils, 20% bands, 29% lymphocytes, and 9% monocytes; haemoglobin concentration 11·2 g/dl and no C reactive protein; and serum IgG concentration 535 mg/100 ml, IgM 98 mg/100 ml, and IgA 11 mg/100 ml. Serum IgM antibodies to *C trachomatis* L₂ reticulate bodies and elementary bodies were detected by enzyme linked fluorescence assay. After 10 days of life she suffered recurrent apnoeic attacks. Treatment was continued with positive end expiratory pressure, and she received 8 ml whole blood. After 40 days of life a tendency to bleed over the whole body developed, and she died of renal failure and pulmonary bleeding. Sections of lung tissue obtained at necropsy were tested by immunofluorescence. Intracytoplasmic inclusions of *C trachomatis* were detected using monoclonal antibodies labelled with fluorescein isothiocyanate (FITC).

*Case 2* The mother of this girl was a 28 year old gravida II para-II. At birth, after 31 weeks and six days of gestation, the girl weighed 1140 g, she was the second child of twins. She was delivered by caesarean section owing to a forelying placenta. The membranes were artificially ruptured at the time of delivery, but the amniotic fluid was clear. After one minute the Apgar score was 7. During the first hours of life retraction and tachypnoea were noted. A chest radiograph showed bilateral reticulonodular changes (Fig. 2). Laboratory tests yielded the following results: white cell count 29·7 × 10⁹/l, with 56% neutrophils, 3% bands, 35% lymphocytes, and 5% monocytes; blood, cerebrospinal fluid, and urine culture yielded negative results; haemoglobin concentration was 15·0 g/dl, and no C reactive protein was found; and serum IgG concentration was 384 mg/100 ml, IgM 34 mg/100 ml, and IgA 24 mg/100 ml. Serum IgM antibodies against *C trachomatis* L₂ reticulate bodies and elementary bodies were detected by enzyme linked fluorescence assay. Treatment was started with continuous positive airway pressure and theophylline. Ampicillin was started intravenously. The baby was kept in the neonatal intensive care unit for 90 days, during which time she remained dyspnoeic.

Aged 1 year and 2 months she died of chronic pulmonary failure. Sections of lung tissues obtained at necropsy were tested by immunofluorescence. Intracytoplasmic inclusions of *C trachomatis* were detected using FITC labeled monoclonal antibodies. At necropsy the lung showed advanced interstitial bleeding, atelectasis, and emphysema.

**Study population**

Sera were obtained from 16 premature infants with chronic respiratory diseases (clinically diagnosed as bronchopulmonary dysplasia or the Wilson-Mikity
syndrome, or both) who had been admitted to Hokkaido Children's Hospital and Medical Center from January 1980 to November 1984 (range 0 to 7 days old). These sera were frozen at −20°C before being tested. Sera were also obtained from 31 neonates with raised serum IgM concentrations but without symptoms of respiratory tract disease. Sera were also collected from 37 premature infants with extremely low birth weights. Sections of lung tissue were obtained at necropsy and biopsy from four premature infants with chronic respiratory disease.

IgM antibodies to *C. trachomatis* L2 reticulate bodies, and elementary bodies were determined by enzyme linked fluorescence assay according to the methods described previously.9,10 The sections of lung tissue were tested for the presence of antigens by direct immunofluorescence, using fluorescein conjugated monoclonal antibodies to *C. trachomatis* that react to the species specific major outer membrane protein of *C. trachomatis*11-13 (Syva, Palo Alto, California).

The samples, which had been embedded in paraffin, were treated with xylol, ethanol (96% vol/vol), and distilled water, each for five minutes.

**Results**

Of the 16 premature infants with chronic respiratory diseases, five had IgM antibodies to *C. trachomatis* L2 reticulate bodies and elementary bodies by enzyme linked fluorescence assay (titre ≥1/500). The serum IgM values of these five infants were higher than those of infants of the same age. Of the 37 sera from premature infants of extremely low birth weight, two had IgM antibodies against *C. trachomatis*. These two infants also had raised serum IgM values and slight symptoms of respiratory disease. The Table summarises the clinical and laboratory findings in the nine premature infants with chronic respiratory diseases with raised serum IgM values. No specific IgM antibodies to *C. trachomatis* were detected in the 31 neonates without respiratory tract symptoms who also had raised serum IgM values. The immunofluorescence test, using FITC conjugated monoclonal antibodies, showed intracytoplasmic chlamydial inclusions in the sections of lung tissues from two premature infants with chronic respiratory disease positive for IgM antibody that had been obtained at necropsy (Fig. 3). Isolation of *C. trachomatis* and determination of antibodies were not carried out on the mothers.

**Discussion**

The Wilson-Mikity syndrome and bronchopulmonary dysplasia are chronic pulmonary disorders.

### Clinical and laboratory findings in premature infants with chronic respiratory diseases and raised serum IgM values

<table>
<thead>
<tr>
<th>Case No</th>
<th>Sex</th>
<th>Gestational age</th>
<th>Birth weight (g)</th>
<th>IgM (mg/100 ml)</th>
<th>IgM antibody to <em>C. trachomatis</em></th>
<th>White cell count (×10⁶/l)</th>
<th>C reactive protein</th>
<th>Premature rupture of membrane</th>
<th>Caesarean section</th>
<th>Identification by monoclonal antibodies</th>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>27 weeks + 0 days</td>
<td>980</td>
<td>98</td>
<td>+</td>
<td>9800</td>
<td>−</td>
<td>−</td>
<td>+ (necropsy)</td>
<td>+ (necropsy)</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>31 weeks + 6 days</td>
<td>1140</td>
<td>34</td>
<td>+</td>
<td>29700</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+ (biopsy)</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>28 weeks + 2 days</td>
<td>1220</td>
<td>121</td>
<td>+</td>
<td>15300</td>
<td>5+</td>
<td>−</td>
<td>+</td>
<td>− (biopsy)</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>25 weeks + 0 days</td>
<td>812</td>
<td>26</td>
<td>+</td>
<td>17400</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>NT (necropsy)</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>27 weeks + 6 days</td>
<td>1180</td>
<td>25</td>
<td>+</td>
<td>11000</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>NT (biopsy)</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>30 weeks + 6 days</td>
<td>1450</td>
<td>51</td>
<td>−</td>
<td>34800</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>− (biopsy)</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>28 weeks + 6 days</td>
<td>1100</td>
<td>196</td>
<td>−</td>
<td>23800</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>− (biopsy)</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>24 weeks + 0 days</td>
<td>630</td>
<td>21</td>
<td>+</td>
<td>26400</td>
<td>4+</td>
<td>−</td>
<td>−</td>
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</tr>
<tr>
<td>9</td>
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<td>30</td>
<td>−</td>
<td>27200</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>− (biopsy)</td>
</tr>
</tbody>
</table>

Normal range for serum IgM is 0–20 mg/100 ml. NT = not tested.
Monoclonal antibodies have especially been detected in infection with Chlamydia trachomatis. Mardh et al'8 reported a case of intrauterine infection with Chlamydia trachomatis in a premature infant born in the 29th week of gestation by caesarean section. These studies confirmed the possibility of intrauterine infection with Chlamydia trachomatis.

IgM antibodies to Chlamydia trachomatis are most likely to be detected in cases of systemic chlamydial infections, especially in infantile pneumonia. In previous studies we reported that enzyme linked fluorescence assay was sensitive and specific for determining IgM antibodies against Chlamydia trachomatis. Furthermore, monoclonal antibodies have recently proved useful for diagnosing Chlamydia trachomatis infection in pediatrics.

In this study we were unable to determine levels of IgM antibodies against Chlamydia trachomatis in the mothers of premature infants. Further studies, including studies of the pathogenic role of Chlamydia trachomatis in the perinatal period, are now in progress.

References


Requests for reprints to: Dr Kei Numazaki, Department of Pediatrics, Sapporo Medical College, S, I W, 16 Chuoku, Sapporo, 060 Japan.
Chlamydia trachomatis.

Premature infants caused by Chlamydia trachomatis.

K Numazaki, S Chiba, K Kogawa, M Umetsu, H Motoya and T Nakao

doi: 10.1136/jcp.39.1.84

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