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Use of counter-immunoelectrophoresis for the detection of chlamydial antigens in serum

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An increasing number of reports on Chlamydia trachomatis pneumonia in infancy has recently been published in the literature. Demonstration of the aetiologic agent depends, however, on laborious culture procedures and serological techniques. Based on the observation of a cross-reaction between certain Acinetobacter species and Chlamydiae, the detection of chlamydial antigens in sera of 13 infants with pneumonia due to Chlamydia trachomatis was performed with antiserum to Acinetobacter by the counter-immunoelectrophoresis technique.

There has been an increasing number of recent reports on Chlamydia trachomatis pneumonia in infancy [1, 2, 5, 6, 7, 8, 10, 14, 20]. Despite a rather distinctive clinical syndrome, the diagnostic tests used for the detection of Chlamydia trachomatis depend on time-consuming and sophisticated procedures like recovery of the organism from the nasopharynx or tracheal secretion, and various serologic techniques. Encouraged by the reports on the detection of antigen in sera of patients with Pneumocystis carinii pneumonitis using the counter-immunoelectrophoresis technique [11, 13], we employed this method for a rapid diagnosis of chlamydial pneumonia in early infancy.

Based on the observation of a serological cross-reaction between Acinetobacter calcoaceticus subspecies anitratus and Chlamydia [3, 4], detection of chlamydial antigens in sera was performed with antiserum to Acinetobacter by counter-immunoelectrophoresis. After a preliminary report [17] we now present our advanced experiences of this technique in 13 infants with Chlamydia trachomatis pneumonia.

Material and Methods

Counter-immunoelectrophoresis (CIE). In our study CIE was performed with an immunoelectrophoresis kit (Instrumentation Laboratory, D-5303 Bornheim 2) containing a universal electrophoresis chamber, a glass slide ($225 \times 75 \times 3$ mm), sodium barbital buffer solution (pH 8.6; ionic strength 0.056), filter paper wicks, a power supply (Pherostat 273) and an agar gel puncher [15]. The glass slide was coated with 25 ml of 1% agarose (Agarose H; LKB Instrument GmbH, D-8032 Gräfelfing) dissolved in the buffer solution.

After cooling, parallel wells 3 mm in diameter were punched 7 mm apart (edge to edge), and the slide was placed in the centre of the chamber. Wells were filled with solution, using capillary pipettes. Antibody containing wells were placed at the anodal side and patients' neat sera (i.e., neither diluted nor concentrated) at the cathodal side. The agarose coated slide was attached by filter paper wisks to reservoirs containing buffer solution. The universal chamber attached to the power source, a constant current of 60 mA (at the power source) was applied for 80 min at room temperature. Slides were inspected unstained for precipitin lines without any additional aid. The production of rabbit hyperimmune serum to Acinetobacter calcoaceticus was carried out by one of us (H. B.); its efficacy for the detection of chlamydial antigens has been published [3, 4].

The potential usefulness of this antiserum for a precipitating reaction with chlamydial antigens in the setting of our CIE technique could be demonstrated using chlamydial group antigens (Institut Pasteur Production, Code 52471), prepared from infected yolk sacs [21]. These antigens served as a positive control during the CIE procedure with patients' sera.

Patients. The study included 48 selected infants admitted from January, 1982, to July, 1983. Their clinical diagnosis (physical findings and radiographic evidence) was either a "delayed" respiratory distress syndrome [18, 19] in older newborns (>one week) with concomitant purulent conjunctivitis and eosinophilia (>400/mm^3) in their blood count, or an afebrile pneumonia with a chronic cough (>one week) in young infants (<six months). The latter had sometimes a staccato cough similar to that with pertussis.

Additional blood samples were obtained from each child for blood cultures and CIE.

For the documentation of a chlamydial infection we determined anti-chlamydial antibody titres. This was also performed by CIE with sera collected from the patients on admission or at the time of the first suspicion of a chlamydial infection, respectively, and with sera after a clinical course of three to four weeks. The source of antigen was the same which had served as a positive control.

The mere qualitative detection of anti-chlamydial antibodies was demonstrated with the patients' undiluted serum samples. Then twofold dilutions of the patients' sera were run against the reference antigen. The highest serum dilution giving a visible precipitin reaction in the CIE procedure was considered the quantitative titre of anti-chlamydial antibodies.

Beside the determination of these titres in all 48 infants, we took secretions of the nasopharynx or trachea of the children with a positive reaction in the CIE procedure (patients' sera and Acinetobacter antiserum) for routine bacteriological cultures and for the demonstration of intracellular chlamydial inclusion bodies in tissue cultures (Mc Coy cells). The latter was done in a two step procedure: First we took the clinical specimen which was immediately frozen to minus 200°C by liquid nitrogen and then stored at this temperature until the final culture procedure with Mc Coy cells [9].

Thirteen newborns with the diagnosis of hyaline membrane disease or aspiration syndrome were selected as control patients.

Results

There were 13 positive results in the CIE procedure of the selected infants. Table I demonstrates the results of the children submitted to Mc Coy cell cultures. All blood cultures were negative for aerobic and anaerobic bacteria. All routine bacteriological cultures from nasopharynx and trachea secretions were negative for Acinetobacter species.

All other children had negative, non-diagnostic titres; there were 11 infants with a qualitative detection of antibodies, but no precipitin reaction with diluted samples.

According to the method applied, the quantitative titres of the anti-chlamydial antibodies did not differentiate between IgM and IgG antibodies. There was, however, an at least twofold increase between the first titres and those obtained three to four weeks later in all 13 children (by this means excluding passively transmitted maternal antibodies). The latter titres are those depicted in Table I.

TABLE I

Results of Mc Coy cell cultures and antigen detection by counter-immunoelectrophoresis in 13 children

Secretions	Culture results (Mc Coy cells)	Routine bacteriological cultures	Counter-immunoelectrophoresis serum	Anti-chlamydial antibody titre
Trachea	+	—	+	1 : 64
Trachea	+	—	+	1 : 64
Trachea	+	—	+	1 : 128
Nasopharynx	+	—	+	1 : 128
Nasopharynx	—	—	+	1 : 32
Nasopharynx	+	—	+	1 : 64
Trachea	—	—	+	1 : 128
Trachea	+	—	+	1 : 64
Nasopharynx	—	—	+	1 : 64
Trachea	+	—	+	1 : 128
Nasopharynx	+	—	+	1 : 64
Nasopharynx	+	—	+	1 : 32
Trachea	+	—	+	1 : 32

The cause for the three negative Mc Coy cell cultures with a positive CIE result could not be evaluated. They might have been due to some technical problem on the way from the patient to the final procedure in the laboratory.

There was no positive CIE result in the 13 control patients; they had no positive blood culture and all Mc Coy cell cultures were negative for chlamydial inclusion bodies. In eight control patients a qualitative detection of chlamydial antibodies was possible, but no precipitin reaction occurred with diluted serum samples.

DISCUSSION

Chlamydia trachomatis pneumonia has recently been described as a

distinctive syndrome characterized by a chronic, afebrile course, diffuse lung involvement, elevated serum immunoglobulins, and eosinophilia. Although these clinical findings apparently are indistinguishable from the pneumonia syndromes associated with other organisms like Cytomegalovirus, Pneumocystis carinii, and Ureaplasma urealyticum [14], diagnostic investigations to demonstrate a specific infectious agent should be made because of therapeutic and prognostic implications. Unfortunately, identification of a specific aetiologic agent depended on rather laborious culture procedures and serologic techniques until recently. CIE has shown promise not only in the early detection of bacterial antigens [12, 15, 16], but also in the recognition of parasitic agents like Pneumocystis carinii [11, 13].

Because culture and isolation of Chlamydia trachomatis are currently available in a few laboratories, diagnostic antisera against its elementary bodies seem difficult to obtain. The observation of a serologic cross-reaction between Acinetobacter calcoaceticus and Chlamydia [3, 4] encouraged us to use this more available hyperimmune serum to bacterial antigens in a CIE setting. Our study not only indicates that chlamydial pneumonias are associated with an antigenaemia of the aetiologic agent, but also demonstrates that a rapid detection of chlamydial antigen is possible in serum using the CIE technique. Based on the findings of this antigenaemia, future developments are to aim at the use of genuine anti-chlamydial antibodies avoiding the still necessary exclusion of a positive blood culture with Acinetobacter species. With the availability of monoclonal anti-chlamydial antibodies still further improvements will probably be obtained with the detection of chlamydial antigens.

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