Electron microscopy and serological features of a patient with Q fever prosthetic valve endocarditis

B J Isalska, A Curry, T N Stanbridge, D Tweddle, E O Caul

Abstract
The clinical, serological and electron microscopic findings in a 47 year old woman with bioprosthetic valve coxiella endocarditis occurring 15 years after streptococcal endocarditis are described. The patient underwent valvular surgery a total of four times to control symptoms and remains well on medical therapy more than two years after her last operation. (J Clin Pathol 1996;49:679-687)

Keywords: Q fever, endocarditis, prosthetic valve.

Q fever infection in humans is a zoonosis acquired through the respiratory route. The disease is often a self-limiting febrile illness, but diverse and more serious presentations include pneumonia, osteomyelitis, and neurological abnormalities. Endocarditis is the most frequent clinical expression (68%) of chronic infection and remains the most frequent lethal complication. The causative agent, Coxiella burnetii, can be grown in tissue culture but the diagnosis usually relies on detection of antibodies. We wish to document the electron microscopy features of a patient with prosthetic valve endocarditis caused by C burnetii, the patient having had native valve streptococcal endocarditis 15 years previously.

Case report
A 47 year old Irish woman was admitted to hospital with a five day history of rigors, night sweats and malaise. She had had rheumatic fever when aged seven years and remained well until the age of 32 years when she developed endocarditis due to viridans streptococci. This was treated with intravenous penicillin, initially as monotherapy and later in combination with streptomycin. She developed a skin rash and her antibiotic treatment was completed with co-trimoxazole.

One year later (1977) the patient underwent homograft aortic valve replacement with mitral valve repair, and then, four years later, a porcine xenograft (Carpentier-Edwards) mitral valve replacement (1981). The histology of the excised valves was consistent with previous bacterial endocarditis. The patient enjoyed good health for 11 years until the present episode (1992). She admitted to being unwell over the past eight months, with hot flushes, lethargy and a 10 kg weight loss. She denied excess alcohol intake. On examination, the patient was febrile (38°C), clinically anaemic, with hepatosplenomegaly and clinical features of mitral and aortic regurgitation. There were no other peripheral stigmata of endocarditis.

Initial laboratory investigations were as follows: haemoglobin 10.6 g/dl with a normochromic, normocytic picture; white cell count 4.2 x 10⁹/l; erythrocyte sedimentation rate 25 mm/hour; C-reactive protein (CRP) 55 mg/l; and an International Normalised Ratio of 4.5. She had abnormal liver function tests (LFTs): alkaline phosphatase 449 IU/l (normal range 30–140); γ-glutamyl transpeptidase 99 IU/l (normal < 35); aspartate amino transferase 126 IU/l (normal 4–40); lactate dehydrogenase 800 IU/l (normal 200–600); but normal protein 66 g/l, bilirubin and urea and electrolytes. Microscopy and culture of urine were negative.

Thoracic echocardiography revealed a mitral valve with thickened, abnormal cusps but no clear vegetation. There was a small paraprosthetic leak and moderate pervalvlar regurgitation but the valve was not rocking. The aortic valve showed moderate incompetence with no dehiscence or abscess. Transoesophageal studies were non-contributory.

The hepatosplenomegaly was confirmed by abdominal ultrasound but no focal abnormality was evident.

Four separate sets of blood cultures, collected before commencement of vancomycin and gentamicin therapy, were negative. The patient became afebrile after several days’ treatment with the antibiotics and with normalisation of her CRP and LFTs. However, raised titres of antibodies to phase I and II C burnetii by the complement fixation test and raised IgG and IgA titres by the immunofluorescence test, confirmed the diagnosis of
Table 1  Results of complement fixation and immunofluorescence tests

<table>
<thead>
<tr>
<th>Date</th>
<th>Complement fixation titre</th>
<th>Immunofluorescence titre*</th>
<th>IgG</th>
<th>IgM</th>
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<tr>
<td></td>
<td>Phase 1</td>
<td>Phase 2</td>
<td></td>
<td></td>
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<tr>
<td>Feb 1993</td>
<td>512</td>
<td>&gt; 512</td>
<td>NT</td>
<td>&gt; 1280</td>
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<tr>
<td>March 1993</td>
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<td>NT</td>
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<tr>
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<td>512</td>
<td>&gt; 512</td>
<td>&gt; 4096</td>
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<tr>
<td>Dec 1995</td>
<td>24</td>
<td>64</td>
<td>NT</td>
<td>1280</td>
</tr>
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</table>

*Reciprocal of the dilution showing positive (distinct) immunofluorescence.  A/C = anticomplementary; NT = not tested.

chronic Q fever infection (table 1). The patient’s treatment was changed to doxycycline and rifampicin, and then to doxycycline and ofloxacin after the development of a rash.

Three months later, the patient required replacement of aortic and mitral valves (1993) because of severe mitral and aortic regurgitation. Light microscopy of the excised valves showed calcification, fibrosis and some histiocytes with giant cells. Prosthetic valve tissue was also processed and examined by electron microscopy (see below). A homogenised suspension of the valves was inoculated into guinea pigs, producing seroconversion to C burnetii complement fixing antigen, confirming the diagnosis of coxiella prosthetic valve infection.

Examination by electron microscopy of the patient’s archived native aortic and mitral valves failed to demonstrate coxiella infection.

**Electron microscopy**

Pieces of prosthetic heart valves were fixed in 5% glutaraldehyde (v/v) made up in 0.1 M cacodylate buffer. Small pieces of tissue/surface vegetation were removed, washed in buffer, post-fixed in 1% osmium tetroxide (w/v), dehydrated in a graded series of alcohols and embedded in Agar 100 resin (Agar Scientific). Ultrathin sections were cut on a Reichert OMU4 Ultracut ultramicrotome, mounted on uncoated 400 mesh copper grids, stained with uranyl acetate and lead citrate and examined in an AEI EM801 electron microscope.

**Results**

Many small rod shaped or coccal bacteria were found within cells of vegetations on the prosthetic mitral valve (fig 1A). No bacteria were found in the extracellular collagenous

![Figure 1](image-url)  (A) Low magnification electron micrograph of cytoplasm from mitral valve vegetation, showing numerous intracellular bacteria. Rod shaped and coccal forms can be seen. The lucent contents of the bacteria suggests that they are non-viable. Some show evidence of calcification (arrowheads) (magnification x8000). (B) Non-dividing, rod shaped organism showing surface layer of fibrillar or granular material, which may be polysaccharide (arrowhead) (magnification x76 000). (C) Coccal forms showing similar ultrastructure to that shown in fig 1B (magnification x65 000). (D) Rod shaped bacteria showing evidence of a division wall. Note the shrunken protoplast within electron-lucent interior of the organism and condensed nature of the nucleoid (magnification x47 500).
matrix. The bacteria were intracytoplasmic but extranuclear in location, although some appeared in shallow indentations of the nuclear envelope. The wall structure was typically Gram negative and composed of an internal plasma membrane and a surrounding membrane-like wall component. External to the outer membrane-like wall component was a roughly 10 nm thick fibrillar or granular medium electron dense layer, which could be indicative of a surface polysaccharide layer (figs 1B and 1C). Some rod shaped organisms showed evidence of division (fig 1D). There was also evidence of damage to most of the bacteria. The internal protoplast had shrunk back within the lucent contents of the outer envelope and the plasma membrane surrounding the protoplast had fragmented, with only a remnant of this membrane being apparent. The bacterial nucleoid was prominent in most bacteria, with fibrils appearing in a thickened and condensed state. Ribosomes were absent in these damaged organisms. The periphery of some organisms appeared very electron dense and internally patchy electron dense deposits could be found, an appearance consistent with early calcification (fig 1A). Rod shaped organisms were 0.6–0.65 μm long and 0.3–0.4 μm wide. Dividing organisms were slightly longer at 1.3–1.4 μm. Coccal forms were 0.4–0.6 μm in diameter.

Subsequent course
Further double valve replacement was undertaken one month later (1993) because of congestive failure and haemolytic anaemia attributed to paraprosthetic leaks. The patient made a good recovery after this further surgery and has remained well ever since, a period of 36 months, being maintained on oral doxycycline and ciprofloxacin.

Discussion
The investigation of patients suspected of having endocarditis, but whose blood cultures remain negative, must include serological screening for C burnetii infection. The incidence of Q fever endocarditis is increasing, although this may reflect increasing recognition of this clinical syndrome. Turck et al reported 16 cases of chronic Q fever diagnosed between 1968 and 1973 and reviewed 55 cases in the world literature. Ninety-two cases were reported in England and Wales between 1975 and 1981, accounting for 3% of all cases of endocarditis reported during that period. In total, about 300 cases have been reported worldwide with a mortality rate ranging between 12 and 23%.2,3 In the vast majority of cases, Q fever endocarditis occurs on previously damaged heart valves.2,3 Duration of symptoms varies from four weeks to four years6 and an environmental source may be found in up to 80%. There are no specific clinical features that help to differentiate it from other causes of endocarditis and therefore diagnosis depends on serological evaluation.

Three serological techniques are available for the investigation of Q fever infection: complement fixation, indirect immunofluorescence, and ELISA. A significant increase in complement fixation titre to the phase II antigen indicates acute infection, whereas persistently elevated titres of phase I antibodies (≥ 200) reflect chronic Q fever infection—for example, endocarditis. The serological differentiation between the late acute infection and endocarditis has been studied and the discriminatory value of the IgA titre against phase I highlighted.7,8 Valve replacement surgery is necessary in over 50% of patients8 and immunohistochemical staining and electron microscopy may demonstrate the organisms. Inoculation of excised infected tissue into animals or propagation in fibroblast cultures may also help to confirm the diagnosis.

Tetracyclines or their derivatives, in combination with either co-trimoxazole or a quinolone, have been the mainstay of antibiotic treatment. Eradication of the infection by bacteriostatic drugs is extremely difficult to achieve and a minimum of three years' treatment has been suggested9 before considering whether to discontinue antibiotic treatment. Preliminary reports of therapy with a combination of hydroxychloroquine and doxycycline are promising.10 While this patient remains well and in the absence of adverse effects from antibiotics, it is proposed that she will continue on long term doxycycline and ciprofloxacin treatment.

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