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# Characterization of the antibody response in *Corynebacterium jeikeium* septicaemias

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SUMMARY

*Corynebacterium jeikeium* causes septicaemia in neutropenic patients usually after colonizing intravenous lines. This paper reports the results of immunoblotting sera from 14 patients with a *C. jeikeium* septicaemia. Recovery from the septicaemia was associated with production of both IgM and IgG against antigenic bands of 50, 52 and 110 kDa. Antibody against the 110 kDa band was present in controls but the antibody against the 50 and 52 kDa was specific to those patients who had on-going or previous *C. jeikeium* infection. A case of *C. jeikeium* endocarditis is also presented and here recovery was associated with seroconversion to the 50 and 52 kDa bands.

## INTRODUCTION

Recent years have seen the description of infection in neutropenic patients due to *Corynebacterium jeikeium* [1]. These microorganisms are characterized by being catalase positive, oxidase negative, fermentative Gram-positive aerobic rods which do not reduce nitrate [2]. They differ from other coryneforms by being highly resistant to antibiotics including ampicillin, cephalothin, chloramphenicol, erythromycin, gentamicin, penicillin G, streptomycin and tetracycline [3]. Conventional therapy for a *C. jeikeium* septicaemia is systemic vancomycin which is potentially nephrotoxic. This mitigates against its blind use in pyrexial neutropenic patients, so that it has become important to develop a marker of *Corynebacterium jeikeium* infection.

The SDS PAGE profiles of 102 strains have previously been compared and found to be largely identical [3]. This paper examines the antibody response in *C. jeikeium* septicaemia and endocarditis; this is dissected out by the technique of immunoblotting so that antibody production against individual antigenic bands can be correlated with survival from infection. This also delineates those antigenic bands where the presence of antibody would suggest previous *C. jeikeium* infection.

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## PATIENTS AND METHODS

### *Patients*

*Controls*

Sera were examined from 18 pyrexial neutropenic leukaemics where all cultures were negative for the JK coryneform; these included blood and those taken from any indwelling intravenous line.

*Septicaemic patients*

Between two and five sequential sera were examined from 14 patients with *C. jeikeium* septicaemias. Thirteen of the 14 cases were neutropenic patients who recovered on vancomycin therapy. Their pyrexia corresponded with a blood culture positive with the *C. jeikeium*. Three of the cases had acute lymphatic leukaemia and the remaining 10 had acute myeloid leukaemia. The fourteenth case was a patient who was admitted to an Intensive Therapy Unit following a road traffic accident. He was not neutropenic and his *C. jeikeium* septicaemia followed colonization of a long-term indwelling central line. He responded to vancomycin therapy.

*Endocarditis patient*

Case 15 was a patient with ulcerative colitis with an intravenous feeding line which became colonized by *C. jeikeium*. The patient subsequently developed endocarditis as judged by a positive echo-cardiogram and continuing pyrexia. Three sets of blood-cultures grew the *C. jeikeium* and the patient gradually recovered after systemic vancomycin therapy.

### *Methods*

*Strain*

A clinical isolate of *C. jeikeium* obtained from a septicaemic case was used throughout. This came from St Bartholomew's Hospital where eight of the septicaemic cases formed a cluster on the oncology ward. In these cases it was felt there would be no variation in antibody response due to strain specific antigens. There was insufficient serum to gauge the response against multiple isolates.

*Preparation of protein extracts*

The isolate was sub-cultured onto Columbia blood-agar (Oxoid) and incubated at 35 °C for 48 h aerobically. The plates were harvested with a loop in distilled water and the resulting cell suspension spun at 6000 g for 20 min. The pellet was resuspended in its own volume of sterile distilled water and placed inside an Xpress. It was crushed at −20 °C and centrifuged at 12000 g for 20 min. The supernatant was used in subsequent experiments and stored at −20 °C. It was standardized to a protein concentration of 10 mg/ml.

*Immunoblotting of patients sera*

After heating with cracking buffer (2·6% sodium dodecyl sulphate, 1·3% 2-mercepto-ethanol, 6% glycerol, 0·2% bromophenol blue, 0·05% M Tris hydrochloride (pH 6·8)) at 100 °C for 2 min, 30 μg of the *C. jeikeium* pressate was

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Table 1. *Details of the antibody responses in control patients*

| Apparent molecular weight (kDa) | IgM | IgG |
|---|---|---|
| 170 | 3 | 3 |
| 154 | 0 | 1 |
| 110 | 4 | 3 |
| 86 | 0 | 1 |
| 70 | 0 | 4 |
| 60 | 1 | 0 |

loaded onto each well of a 10% polyacrylamide gel. Electrophoresis and transblotting were performed as described previously [4, 5]. The gel was transferred onto a nitrocellulose membrane in an LKB transblotter (LKB laboratories). The buffer contained methanol 20%, 25 mM Tris and 192 mM glycine at pH 8·3 and transfer was allowed to proceed at 25 °C for 45 min. The nitrocellulose paper was blocked in bovine-serum albumen 3% in buffered saline (NaCl 0·9% and 10 mM Tris, pH 7·4) at 4 °C overnight. The nitrocellulose was then incubated at 25 °C for 2 h with the human serum diluted 1:10 in buffered saline containing bovine serum albumin 3% and Tween 20 0·05%. After washing five times for 30 min in saline 0·9% and Tween 20 0·05%, the nitrocellulose was incubated for 1 h at 25 °C with alkaline phosphatase conjugated goat anti-human immunoglubulin M (IgM) or IgG (Sigma Chemical Co.). After washing again, the membranes were incubated for 15 min at 25 °C with buffer (100 mM Tris hydrochloride pH 9·5, 100 mM-NaCl, 5 mM-$MgCl_2$) containing a mixture of 66 $\mu$l per 10 ml of nitro-blue tetrazolium (NBT 50 mg per ml in *N,N*-dimethylformamide 70%) and 33 $\mu$l per 10 ml of 5-bromo-4-chloro-3-indolylphosphate (BCIP 50 mg/ml in *N,N*-dimethylformamide 70%). The reaction was stopped by washing in water. All the immunoblots were examined and split into trace responses where a reflectance densitometer produced a trace with a height of less than 40 mm, and positive where the height was greater than 40 mm.

Molecular weight markers were Prestained Rainbow Markers (code RPN.756 Amersham International). These were myosin, 200 kDa; phosphorylase b, 92·5 kDa; bovine serum albumin, 69 kDa; ovalbumin, 46 kDa; carbonic anhydrase, 30 kDa; trypsin inhibitor, 21·5 kDa; and lysozyme, 14·3 kDa.

## RESULTS

The results from the 18 control patients are given in Table 1. Six bands were detected and the commonest antibody response was to the band at 110 kDa. Sequential sera were available in four of the patients where there was an antibody response and the patterns did not change over a period of at least 2 weeks. Trace and positive results were combined for this table.

### *Septicaemic patients*

The results from these patients are summarized in Tables 2 and 3. They are given according to four criteria. These are: a constant trace reaction, a constant positive reaction, an increase in brightness which is at least a doubling of intensity

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Table 2. *Details of the IgM response in septicaemic patients to individual* C. jeikeium *bands*

| Apparent molecular weight (kDa) | IgM | | | |
|---|---|---|---|---|
| | Constant | | Increase in brightness | Appearance of band |
| | Trace | Positive | | |
| 170 | 4 | 8 | 2 | |
| 160 | 2 | 2 | 1 | 1 |
| 158 | 3 | 1 | 1 | 1 |
| 154 | 3 | 1 | | |
| 148 | | 2 | 1 | |
| 110 | 3 | 3 | 5 | 3 |
| 86 | | 1 | | |
| 80 | | 1 | | |
| 70 | | 2 | | |
| 65 | | 2 | | |
| 60 | | | | |
| 57 | 1 | | | 1 |
| 52 | 2 | | | 7 |
| 50 | 1 | | | 3 |
| 43 | 1 | | | |
| 40 | | | | |

Table 3. *Details of IgG response in septicaemic patients to individual* C. jeikeium bands

| Apparent molecular weight (kDa) | IgG | | | |
|---|---|---|---|---|
| | Constant | | Increase brightness | Appearance of band |
| | Trace | Positive | | |
| 170 | 5 | 5 | 2 | 1 |
| 160 | 1 | 5 | 2 | |
| 158 | 2 | 2 | 1 | 1 |
| 154 | 2 | 2 | | |
| 148 | 1 | 1 | | |
| 110 | | 2 | 7 | 5 |
| 86 | 3 | 2 | | |
| 80 | 3 | 3 | | |
| 70 | 4 | 2 | | 2 |
| 65 | 3 | | 2 | 1 |
| 60 | 1 | | | |
| 57 | 1 | 1 | | |
| 52 | | | | 14 |
| 50 | | | | 9 |
| 43 | 1 | 1 | | |
| 40 | 1 | 1 | | |

as measured by reflectance densitometry and finally the appearance of a new antigenic band. The results from the serum taken prior to *C. jeikeium* infection are compared to those after infection. IgM levels (Table 2) were static for the bands at 154, 86, 80, 70, 65, 60 and 43 kDa. Two patients showed changes in the bands at 170, 160, 158, 148 and 57 kDa. An increase in or the appearance of IgM against

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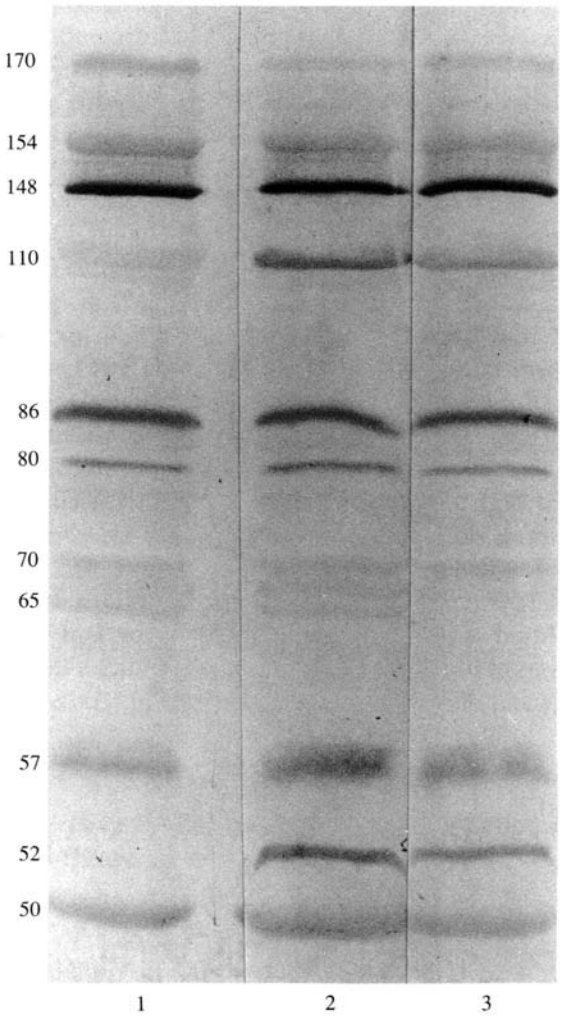


Fig. 1. Sequential immunoblots of the IgG response to the JK coryneform before (track 1), after (track 2) and 5 days after (track 3) a *C. jeikeium* septicaemia.

the bands at 110, 52 and 50 kDa were the most marked changes which occurred during infection. IgG levels (Table 3) showed a similar picture to IgM. Twelve patients developed a more pronounced antibody response to the band at 110 kDa. All the patients upon recovery produced IgG against the band at 52 kDa and an example of this is illustrated in Fig. 1. This patient was the road traffic accident case and also showed an increase in the serum level of IgG against the band at 110 kDa. Track 1 is prior to septicaemia whilst tracks 2 and 3 were taken, respectively, just after the cessation of vancomycin therapy and 5 days later. Nine of the 14 patients also produced IgG against the 50 kDa band.

The case of endocarditis (Fig. 2) had IgM and IgG against all the antigenic bands previously described for the septicaemic patients. The IgM response faded on successful treatment whilst the IgG increased to the 50 and 52 kDa bands.

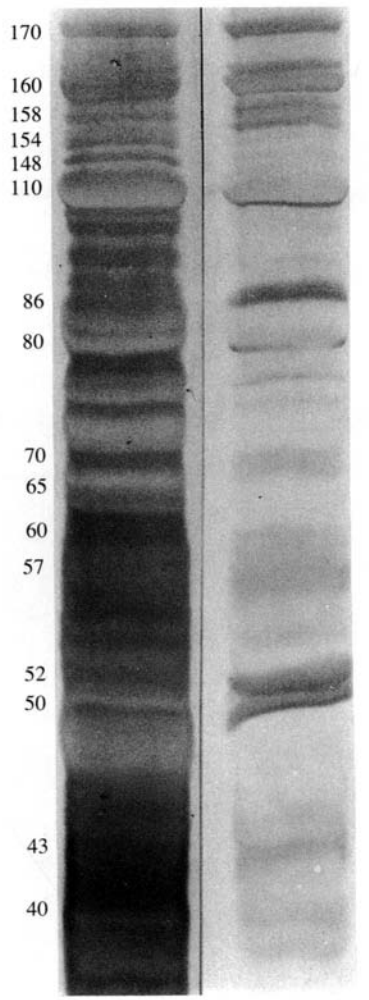


Fig. 2. the IgM (track 1) and IgG (track 2) of the patient with *C. jeikeium* endocarditis against the JK coryneform.

## DISCUSSION

*Corynebacterium jeikeium* has increasingly been recognized as a cause of septicaemia in the neutropenic patient [6, 7]. The current series confirms this in that 13 of the 15 patients were neutropenic at the time of illness. It has previously been reported as secondary and associated with intravenous line colonization [6, 8–10]. All of the patients in this series had long term indwelling lines and in the 10 patients where the results of swabs were available these were positive for the *C. jeikeium*.

The two non-neutropenic patients had cultural evidence of line colonization prior to bacteraemia. In case 15 there was also endocarditis which resolved with vancomycin therapy. The association between *C. jeikeium* infection and endocarditis has been previously reported [11]. In this patient there was antibody against all 16 *C. jeikeium* antigenic bands (Fig. 2). This level of activity was never

seen in the purely septicaemic cases. An analogous situation is seen in streptococcal and enterococcal endocarditis [12]. Here septicaemia can also be differentiated from endocarditis by the number of antigenic bands detected on the immunoblot. Response to therapy correlated with a reduction in the level of serum IgM. In case 15 clinical cure matched a reduction in IgM against all the antigenic bands. The IgG to the bands at 50 and 52 kDa increased indicating a seroconversion to these bands.

The high degree of homogeneity of *C. jeikeium* isolates as judged by SDS-PAGE and DNA-DNA hybridization [11] made it appropriate to study the antibody response in neutropenia against a single clinical isolate. The most striking responses were the antibody changes against the bands at 50, 52 and 110 kDa. Antibody against the 110 kDa antigen either appeared or increased in IgM and/or IgG in 12 of the septicaemic patients. It was widespread in controls (Table 1) which invalidates its use in a serodiagnostic test. Antibody against the 50 and 52 kDa antigenic bands was specific to invasive disease. The 52 kDa band produced the most interesting result with a sequential IgM to the IgG response in nine cases. The remaining five cases were negative on immunoblot for IgM but showed an IgG response. This antigen could form the basis of a serodiagnostic test. The presence of IgM would suggest on-going infection whilst recovery would be associated with an IgG response. The *C. jeikeium* is highly resistant to most commonly prescribed antibiotics so that the test would help rationalize the use of vancomycin in pyrexial, culture-negative, neutropenic patients.

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