Letters to the Editor

High dose immunoglobulin treatment for AIDS related complex (ARC) in haemophilia

A 32 year old HIV antibody positive haemophiliac on home treatment with factor VIII concentrate developed symptoms of AIDS related complex (ARC), with a weight loss of two stone in 10 months, swinging fever, acneiform skin rash, generalised lymphadenopathy, modest thrombocytopenia (platelet count, 112 × 10⁹/l) and lymphopenia (lymphocyte count 0.8 × 10⁹/l).

A bone marrow aspirate showed occasional atypical lymphocytes; a lymph node biopsy specimen confirmed reactive hyperplasia. A polyclonal rise in immunoglobulins was noted. Lymphocyte marker studies showed a reversed T₄:T₈ (suppressor) ratio of 0.32 (normal range = 1.0–2.4). Abdominal computed tomography scan confirmed extensive intra-abdominal lymphadenopathy and splenomegaly. Despite intensive screening no infective cause was found for his fever and there was no response to antibiotics or antiviral treatment. Empirical treatment with high dose intravenous immunoglobulin (sandoglobulin 0.4 g/kg/day) was given to arrest further deterioration.

Within two days, the palpable lymphadenopathy decreased and his fever settled. Full blood counts showed progressive improvement in platelet counts (241 × 10⁹/l maximal on day 13) and lymphocyte counts (1.9 × 10⁹/l maximal on day 10). A computed tomography scan showed persistent splenomegaly, but a striking improvement in previously noted intra-abdominal lymphadenopathy.

Immunoglobulin infusions have been used on HIV positive haemophiliacs with reversed T₄:T₈ ratios, although much of the work has been concerned with in vitro cell function. Four of seven children with AIDS or ARC showed modified in vitro suppressor T cell function following intravenous immunoglobulin infusions.¹

Another haemophiliac with idiopathic thrombocytopenic purpura (ITP) and impaired cellular immunity was treated with high dose immunoglobulin infusions which resulted in an improvement in platelet count and a correction of a low and reversed T₄:T₈ ratio to within normal limits.²

There is, however, little comment on what happened to the patients' well being following treatment in these reports. Our patient had progressive ARC, but showed a remarkable clinical improvement after immunoglobulin, which also preceded a reversal of his laboratory abnormalities. High dose immunoglobulin may therefore be beneficial in the management of other similar patients.

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References


Effect of inoculum size on detection and recovery of Clostridium difficile in selective broth cultures

Clostridium difficile is the causative agent of pseudomembranous colitis and many cases of antibiotic-associated diarrhoea.¹ Although the development of a selective medium (CCFA)² has allowed C difficile to be isolated in the laboratory, many routine diagnostic laboratories have difficulties with reliable detection. We recently advocated the use of a selective enrichment broth for increasing isolation rates of C difficile from clinical specimens.³ Other investigators, however, have reported that enrichment in antibiotic-containing broth cultures does not yield significantly more isolates of C difficile.⁴ In this report we show that detection and recovery of C difficile from selective enrichment broth cultures depends on the size of the inoculum.

A total of eight stool samples known to contain C difficile were obtained from the department of microbiology's clinical laboratory. Gentamicin, cycloserine, cefoxitin (GCC) broths were prepared in 10 ml volumes. Various amounts of a 25% suspension of stool sample in phosphate buffered saline (pH 7.3) were inoculated into the GCC broths. After 48 hours of incubation at 37°C growth and recovery of C difficile were assessed in two ways. The amount of isocaproic acid produced in each broth was determined by gas liquid chromatography, as described previously,¹ and one drop (0.02 ml) of broth culture was inoculated on to CCFA and streaked in the standard manner for isolated colonies. After incubation for 48 hours at 37°C in a Gaspak jar growth was scored on a scale of 0–4 representing no growth to heavy growth.

The effect of inoculum size on the growth and recovery of C difficile from GCC broth cultures is shown in the table. Using a 25% suspension of stool the optimum inoculum size appeared to be between 0.1 ml and 0.2 ml. When larger volumes of 0.5 ml and 1.0 ml were used, growth and recovery, measured by the production of isocaproic acid and by growth on CCFA, respectively, were greatly reduced. With some samples, isocaproic acid was not detected by gas-liquid chromatography, using an inoculum of 1.0 ml, but the GCC broth yielded a few colonies of C difficile after subculture on to CCFA. The difference in the amount of isocaproic acid detected in GCC broth cultures inoculated with 0.1 ml or 0.2 ml stool suspension compared with 0.5 ml of stool suspension was not significant. There was, however, a significant difference between an inoculum of 0.1 ml or 0.2 ml and an inoculum of 1.0 ml of stool suspension (p < 0.01, Student’s t test).

There was an inverse correlation between recovery and inoculum size. There may be several reasons for these findings. Aeration of the GCC broth with the introduction of larger inocula may have slowed the growth of C difficile. The pre-reduced supplemented brain heart infusion broth, however, which forms the basis of GCC broth, is a relatively poised medium and quickly returns to a reduced state. The dilution factor may have also had some effect, as adding 1.0 ml of inoculum would have reduced the concentrations of the antibiotics by 8–9%. In particular, the activity of cefoxitin may have suffered as many enteric facultative Gram-negative bacilli have minimum inhibitory concentrations of cefoxitin in the range 6–8

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Table: Effect of inoculum size on detection and recovery of Clostridium difficile in 10 ml of GCC broth

<table>
<thead>
<tr>
<th>Inoculum (ml)</th>
<th>Isocaproic acid†</th>
<th>Culture‡</th>
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<tbody>
<tr>
<td>0.1</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>0.2</td>
<td>9–25</td>
<td>16.1</td>
</tr>
<tr>
<td>0.5</td>
<td>15–25</td>
<td>16.5</td>
</tr>
<tr>
<td>1.0</td>
<td>1–20</td>
<td>12.2</td>
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</tbody>
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†The inoculum was a 25% suspension of stool in phosphate buffered saline (pH 7.3) expressed as integrated units (height × width of peak).

‡no growth; 1 = scanty growth; 2 = light growth; 3 = moderate growth; 4 = heavy growth.
mg/l. The addition of larger amounts of proteinaceous material would probably have also adversely affected the GCC broth. Undigested vegetable matter could have impaired the action of both selective antibiotics, again permitting the growth of facultative and obligate anaerobic enteric organisms. Several of these organisms have been shown to inhibit the growth of C. difficile in vitro.

All of these factors, to a greater or lesser extent, probably simultaneously play a part in reducing the efficiency of GCC broth when a large inoculum is used. It is important that workers in routine clinical laboratories are aware that it is possible to "overload" selective enrichment broths containing antibiotics. Accordingly, therefore, we would recommend that the maximum inoculum size for 10 ml of GCC broth should be either 0.1 ml or 0.2 ml of a 25% suspension of stool.

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References

Urease activity of Campylobacter pylori

Campylobacter pylori, first cultured in 1982, is increasingly being associated with gastritis and peptic ulceration. Unlike most campylobacters, it possesses a powerful extracellular urease activity. In acute C. pylori gastritis stomach juice urea falls and ammonia rises, with an accompanying rise in pH. The cytopathic effect observed in gastric epithelial cells may be mediated by high local concentrations of ammonia. We decided to investigate this enzyme activity.

Colonies of C. pylori were scraped off blood agar plates, suspended in phosphate buffered saline, then centrifuged. The supernatant was used as the source of urease activity. Aliquots were incubated with urea in buffer, and liberated ammonia measured colourimetrically by the method of Berthelot.

A pH profile showed two pH optima in each of three strains examined, at 5 and at 8. Activity was irreversibly inhibited at and below pH 4–5. The low pH activity was inhibited by phosphate ions, considerably at 10 mM, and almost completely at 250 mM, leaving the pH 8 activity almost unaffected. Both activities were inhibited by low concentrations of acetoxyhydrionic acid; kinetics suggested non-competitive inhibition of each.

We suggest the existence of two extracellular isoenzymes of urease produced by this organism. The low pH one may represent a partial adaptation to the acid environment of the healthy stomach. Hydroxamic acid derivatives have been used therapeutically in man; perhaps they may have some role in controlling the gastritis associated with C. pylori infection, particularly if newer derivatives escape the suspicion of tetracycline and carcinogenicity suggested with older ones.

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References

Dipstick screening for bacteriuria

Boreland and Stoker recently reported the results of a valuable study of the use of dipstick analysis. Screen urines from children for bacteriuria. After studying their report we cannot agree with their conclusion that the method described is suitable for routine use.

The reference culture method was the screening technique using blotting paper strips described by Leigh and Williams. This was originally shown to be suitable for screening large groups of patients who may have asymptomatic infections such as pregnant women. It has not been shown to be suitable for use with specimens from symptomatic patients. In these cases a clinically confined method should be used—for example, one using calibrated loops. Boreland and Stoker did not indicate that their population was predominantly asymptomatic: 12% of their urines yielded significant growth compared with 5% of those studied by Leigh and Williams.

When strips yielded between five and 30 colonies Leigh and Williams recommended that repeat specimens of urine should be examined, as over 40% of the repeat specimens they tested contained more than 10⁸ organisms/ml. Boreland and Stoker gave no consideration to the problem of using with borderline colony counts.

The screening methods using dipstick analysis showed good predictive values for a negative result, but a positive result in a child's urine is important because of the possible consequences of urinary tract infection in childhood. Of the 700 specimens examined, 14 yielded positive cultures, but were negative by the reagent strip methods. Thus 17% of culture positive specimens were not detected by dipstick screening. Concentrating on developing a method which detects negative urines well, the authors seem to have overlooked the clinical importance of paediatric bacteriuria.

The need for bacteriological examination of urine does place a large burden on laboratories, but in attempting to relieve this burden the importance of the results of such examination must not be overlooked.

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References