Letters to the Editor

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

A 32 year old HIV antibody positive haemophilic 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, $112 \times 10^9/l$) and lymphopenia (lymphocyte count $0.8 \times 10^9/l$).

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 (helper): 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 ($241 \times 10^9/l$ maximal on day 13) and lymphocyte counts ($1.9 \times 10^9/l$ 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 haemophilic 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

- 1 Gupta A, Novick BE, Rubinstein A. Restoration of suppressor T-cell functions in children with AIDS following intravenous gamma globulin treatment. *Am J Dis Child* 1986; 140:143-6.
- 2 Zeitlhuber U, Haschke F, Püspök R, et al. Haemophilia and thrombocytopenia in a patient with impaired cellular immunity. *Blut* 1984;48:393-5.

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) broths³ 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,³ and one drop (0.02 ml) of broth culture was inoculated on to CCFA and streaked in the standard manner

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

Inoculum* (ml) (n = 8)	Isocaproic acid†		Culture‡	
	Range	Mean	Range	Mean
0.1	9-25	16.1	2-4	3.2
0.2	15-25	16.5	2-4	3.1
0.5	1-20	12.2	1-4	2.6
1.0	0-20	8.2	1-3	1.9

*The inoculum was a 25% suspension of stool in phosphate buffered saline (pH 7.3)

†Expressed as integrated units (height × width of peak)

‡0 = no growth; 1 = scanty growth; 2 = light growth; 3 = moderate growth; 4 = heavy growth

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 broths 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 of 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