Cytopathic effects of Campylobacter pylori urease

It is now accepted that there is a close association between Campylobacter pylori and gastroduodenal disease,1,2 but the precise nature of the association remains unclear, and the potential pathogenic role of the organism requires investigation. Many possible virulence mechanisms merit consideration including direct toxic effects of bacterial products on cells. We investigated the action of bacteria free preparations derived from C pylori on cell cultures and obtained evidence that urease may play an important part in cell damage.

Three isolates of C pylori from gastric antral biopsy specimens from separate patients in this hospital were used. Organisms were grown on 10% blood agar for 48 hours, suspended in phosphate buffered saline, and centrifuged at 7000 g for 20 minutes. The supernatants were filtered using a 0-2 µm filter and applied to established Vero cell monolayers. Cytopathic effects were then observed.3

None of the three preparations produced a clearly discernable cytopathic effect after incubation with cells over 96 hours, but when urea was added to the system (30 mmol/l), the cells rounded up within 90 minutes (table) and subsequently lysed. These effects were accompanied by a pronounced rise in pH. The three C pylori preparations contained urease, and similar cytopathic effects were obtained using Jack bean and Bacillus ureases (Sigma) in the presence of urea. A two-fold dilution series of ammonia added directly to cell monolayers resulted in the same characteristic cytopathic effect at final concentrations of 1:35 mmol/l and above. If the ammonia was pre-neutralised to give a pH of 7-4 the cytopathic effect was retained at concentrations of 2:7 mmol/l and above. Raising the pH using NaOH produced an entirely different cytopathic effect. These findings support the view that the cytopathic effect produced by the C pylori preparations was related to the generation of ammonia by ureolytic activity and that this effect was largely independent of pH.

The cytopathic activity of our preparations withstood a temperature of 56°C for 15 minutes but was abolished at 80°C. Under the conditions used this activity was not affected by the addition of the competitive urease inhibitor thiourea, which, in contrast, did inhibit the cytopathic effect produced by the commercially available purified ureases. The addition of serum from a patient colonised with C pylori and with high titres of circulating antibodies against the organism, determined by ELISA,4 caused a substantial reduction in cytopathic effect titre (table). This serum had no analogous neutralising effect on the two commercially obtained ureases.

Our findings suggest that the urease activity of C pylori can cause cytopathic effects by the production of ammonia. Although other workers have suggested an important role for urease we have shown directly the cytopathic potential of this activity. As we have also shown that concentrations of ammonia as low as 2:7 mmol/l can produce clear cytopathic effect even at physiological pH, it is likely that local ammonia production by this organism is sufficient to produce cell damage and result in inflammation. We conclude that the ureolytic activity of C pylori may be important in the pathogenesis of gastritis and peptic ulcer.

References