Letters to the Editor

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Cytopathic effects of Campylobacter pylori urease

It is now accepted that there is a close association between Campylobacter pylori and gastroduodenal disease,1 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 bacterial 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.1

None of the three preparations produced a clearly discernible 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 withstand 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 urease5 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 a 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

Table Titres of cytopathic effects produced by C pylori preparations after 20 hours’ incubation with Vero cells under different conditions

<table>
<thead>
<tr>
<th>Test preparation</th>
<th>Nil</th>
<th>30 mM urea</th>
<th>30 mM urea + serum*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C pylori 1</td>
<td>1:384</td>
<td>1:96</td>
<td></td>
</tr>
<tr>
<td>C pylori 2</td>
<td>1:48</td>
<td>1:12</td>
<td></td>
</tr>
<tr>
<td>C pylori 3</td>
<td>1:384</td>
<td>1:96</td>
<td></td>
</tr>
</tbody>
</table>

*added at 1:32 final dilution.

Other correspondence

Brown fat and sudden death

We were interested by the report of brown fat necrosis found in post-perinatal necropsy specimens by Stephenson and Variend.1 Brown fat is a favoured substrate for various virus infections in newborn mice infected with group B Coxsackie viruses.2 Brown fat necrosis can also be produced in mice infected with some group A Coxsackie viruses,3 particularly Coxsackie A7 virus which can do so in adult cotton rats.4 It was for this
reason that suprarenal fat (to include brown fat) was included among the tissues from cases of sudden, unexplained death in infancy which we had virologically tested; both enteroviruses and adenoviruses were isolated from these fat samples, but there were no parallel histological studies. It would have been interesting to have virological data in the cases reported by Stephenson and Variend, especially as the diagnoses of their cases 2, 4, and 8 could well have resulted from virus infections. A combined virological and histological investigation might be worth while.

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References


Giant cell myocarditis associated with lymphoma

In their immunocytochemical study of giant cell myocarditis associated with lymphoma, Hales, Theaker, and Gatter made the interesting observation that necrotic cardiac myocytes did not express desmin reactively. In a recent study of skeletal muscle necrosis and regeneration in the rat we observed a similar loss of desmin expression 24 hours after injection of the local anaesthetic bupivicaine (fig 1). Loss of staining for desmin (using the antibody DE-R-11 from Dakopatts) occurred in fibres which were morphologically normal but which, from evidence in muscles sampled at later dates, were destined to undergo necrosis and phagocytosis. Desmin expression is also absent in necrotic fibres in human myocarditis (fig 2). It would seem that desmin expression, at least as shown by the antibody DE-R-11, is lost at an early stage of skeletal muscle fibre degeneration. The immunohistochemical detection of this loss of expression may provide a sensitive indication of muscle damage in diagnostic specimens.

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References


Dr Theaker comments:
I have investigated this further and have confirmed that desmin expression is lost from cells showing myocytolysis in other...