Letters to the Editor

*PG Kennedy,†Eleanor J Bell,‡Sheila A Keane,¶Morag C Timbury
*Department of Neurology,
†Enterovirus Reference (Scotland)
‡Southern General Hospital,
¶Glasgow, G51 4TF

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 withstanded 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 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.

MR Barer,* TJS Elliott,† DBEERLEY,‡ JE Thomas,‡ EJ Eastham,
††Departments of *Microbiology, ‡Clinical Microbiology, and ‡‡Child Health,
University of Newcastle upon Tyne Medical School,
Framlington Place,
NE2 4HH.

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>Additions</th>
<th>30 mM urea</th>
<th>30 mM urea + serum*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C pylori 1</td>
<td>Nil</td>
<td>1:384</td>
<td>1:96</td>
</tr>
<tr>
<td>C pylori 2</td>
<td>Nil</td>
<td>1:48</td>
<td>1:12</td>
</tr>
<tr>
<td>C pylori 3</td>
<td>Nil</td>
<td>1:384</td>
<td>1:96</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 Viemri.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 adenviruses 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.

NR GRIST*
GED URQUHART

*Communicable Diseases (Scotland) Unit,
Ruchill Hospital, Glasgow, G20 9NB.
Microbiology Department, Ninewells Hospital, Dundee, DD1 9SY.

References


Dr Stephenson and Variend comment:
We thank Grist and Urquhart for their interesting comments. We were unaware of the reports relating to an affinity between virus and brown adipose tissue. This was despite a careful search of published data carried out before our study.

Virological studies are carried out routinely in our cases but these are limited to immunofluorescence of lung tissue for respiratory syncytial virus, parainfluenza I and II; additionally, a stool specimen is examined electron microscopically and by culture. In the miscellaneous group showing brown fat necrosis rotavirus was found in the stool in case 6 and histological assessment in cases 2 and 8 did suggest viral infection of some of the tissues. Two cases of unexplained death with brown fat necrosis showed cytomegalovirus inclusions limited to the parotid gland.

Grist and Urquhart suggest a combined virological and histological investigation of the brown fat. A great number of cases, however, would need to be studied before a reasonable conclusion could be drawn in view of the apparent low incidence of brown fat necrosis. Immunostaining for virus in the tissue sections of the affected cases would seem a better prospect. We do not know, however, whether appropriate antibody for the viruses in question is available, nor indeed whether it could be applied to paraffin sections. If Grist and Urquhart are in a position to assist us in this regard we should be extremely grateful.

Giant cell myocarditis associated with lymphoma

In their immunocytochemical study of giant cell myocarditis associated with lymphoma1 Hales, Theaker, and Gatter made the interesting observation that necrotic cardiac myocytes did not express desmin reactivity. In a recent study of skeletal muscle necrosis and regeneration in the rat2 we observed a similar loss of desmin expression 24 hours after injection of the local anaesthetic bupivacaine (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 myopathies3 (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 indicator of muscle damage in diagnostic specimens.

TR HELLIWELL*
RHT EDWARDS
Departments of *Pathology and Medicine,
University of Liverpool,
Royal Liverpool Hospital,
PO Box 147,
L69 3BX

References


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

Fig 1  Rat tibialis anterior muscle 24 hours after bupivacaine injection. Damaged fibres (*) show loss of desmin expression while adjacent normal fibres show peripheral ring of staining for desmin. (Immunoperoxidase.)
Brown fat and sudden death.

N R Grist and G E Urquhart

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