Identification of a 29 kDa flagellar sheath protein in *Helicobacter pylori* using a murine monoclonal antibody

Catherine J. Luke† and Charles W. Penn

The membrane-like flagellar sheath of *Helicobacter pylori* is of unknown function and little is known of its composition. A murine monoclonal antibody to *H. pylori*, designated GF6, which reacts by immunoblot with a polypeptide with an apparent molecular mass of 29 kDa was shown by immunogold-electron microscopy to label specifically the flagellar sheath structure. The antigen was detected by immunoblot using the monoclonal antibody in all 11 strains, of diverse geographic origin, so far tested. The antibody also reacted weakly with polypeptides with apparent molecular masses of 65 kDa in *Vibrio cholerae* and *Vibrio parahaemolyticus*. The antigen was shown by one- and two-dimensional electrophoretic analysis and immunoblotting to be distinct from the abundant urease subunit UreA, of similar molecular mass. Identification of this flagellar sheath polypeptide will facilitate investigation of the structure and function of the flagellar sheath of this important gastric pathogen.

**Keywords**: *Helicobacter pylori*, flagellum, flagellar sheath

INTRODUCTION

*Helicobacter pylori* is now generally acknowledged to be a major cause of gastritis in man, and is associated with the occurrence of peptic ulceration, particularly in the duodenum (for reviews, see Blaser, 1990, 1993). *H. pylori* infection has also been implicated as a pre-disposing factor to changes leading to gastric carcinoma (Parsonnet et al., 1991; Forman et al., 1991).

Despite the strong pathological associations and extensive clinical information on the occurrence and natural history of this organism, virulence factors and pathogenic mechanisms remain generally unclear. Several potential virulence factors have been identified and investigated. The highly active urease, one of the most potent bacterial ureases known (Ferrero et al., 1988), appears to be essential for colonization in animal models, based on the reduced infectivity of urease-negative mutants (Eaton et al., 1991; Eaton & Krakowka, 1994). Haemagglutinins and adhesins have been investigated since the organism was first isolated, and several classes of adhesins (Doig et al., 1992; Fauchere & Blaser, 1990), including some which appear to utilize sialic acid receptors (Evans et al., 1988; Lewalaguruge et al., 1993), have been described. Toxic activity of the organism has also been examined, and vacuolating cytotoxins are produced by about 50–60% of isolates (Leunk et al., 1988, 1990; Cover et al., 1990). This activity is associated with proteins of 87 kDa (Cover & Blaser, 1992), now shown to be a product of the toxin-encoding *vacA* gene (Schmitt & Haas, 1994; Phadnis et al., 1994), and 120–140 kDa (Apel et al., 1988; Covacci et al., 1993; Tummuru et al., 1993), not the toxin itself but the product of the cytotoxin-associated gene *cagA*. Motility and the expression of flagella are also aspects of the organism which have been implicated in pathogenicity (Eaton et al., 1989). Despite these efforts, a clear identification and characterization of all essential virulence factors of the organism is still lacking. Further investigation is needed on all these fronts.

The role of flagella and motility in bacterial virulence has been the subject of much speculation (reviewed by Penn & Luke, 1992). There are at least two ways in which flagella may contribute: either by conferring motility on the organism, so that it may, for example, be able to penetrate mucus or undergo chemotactic responses towards favourable environments; or by acting in itself as
an adhesin, such that flagellar components might bind to host cell surfaces and contribute to the ability of the organism to colonization epithelia. As examples, non-motile mutants of *Vibrio cholerae* (Guentzel & Berry, 1975) and *Campylobacter jejuni* (Morooka et al., 1985) show diminished virulence. For the latter organism, recent evidence suggests that the flagellar structure itself, rather than motility *per se*, may be the essential component for colonization and invasion in the host intestinal tract (McSweegan & Walker, 1986; Wassenaar et al., 1991). In *H. pylori*, there is as yet no clear evidence whether it is simply the possession of flagella or the expression of motility that is important in virulence.

The flagella of *H. pylori*, composed mainly of a major flagellin of 51–56 kDa (Newell, 1987; Geis et al., 1989; Luke et al., 1990; Kostrynska et al., 1991) with a calculated molecular mass, predicted from the sequence after cloning the flagellin gene, of 53 kDa (Leying et al., 1992), are encased in a membrane-like sheath of unknown function. Such structures are quite rare among motile bacteria (Penn & Luke, 1992), although they also occur in *V. cholerae* (Fuerst & Perry, 1988), swimmer-phase *Vibrio parahaemolyticus* (McCartier & Silverman, 1990), *Beneckea* sp. and *Photobacterium fischeri* (Allen & Baumann, 1971), and *Bdellovibrio bacteriovorus* (Thomashow & Rittenberg, 1985). In most cases the composition of these sheaths is unclear, although some evidence for the presence of outer-membrane components and characteristic proteins has been obtained (Fuerst & Perry, 1988). In *H. pylori*, we tentatively identified proteins of 80, 66, 62, 58, 30 and 26 kDa as potential sheath components (Luke et al., 1990). These proteins were removed from crude flagellar preparations by the detergent Triton X-100. By purifying intact sheaths, following removal of flagella from cells by shearing and dissociation and removal of the flagellar filaments within the sheaths under acid conditions, Geis et al. (1993) showed that proteins of 150, 72, 67, 51, 49 and 29-5 kDa were present in the remaining sheaths. These approaches to analysis of the sheath are dependent on solubilization or retention of all sheath components under the conditions employed. Thus, confirmation of the presence of the individual proteins in situ in the sheath is also needed. We now describe a monoclonal antibody reactive by immuno blot with a protein of 29 kDa (and distinct from the small subunit of urease, UreA, which has a similar molecular mass). The antibody shows by immuno-electron microscopy the location of this protein in the flagellar sheath.

**METHODS**

**Bacterial strains and culture conditions.** *Helicobacter pylori* strains NCTC 11637 (type strain) and Roberts (derived from a clinical isolate originally obtained in Manchester, UK – Luke et al., 1990) were routinely used. Cultures were grown on Isosensitest agar (Oxoid) supplemented with 2.5% (v/v) newborn calf serum (NCS; Life Technologies) or on chocolate agar made with Columbia agar base (Difco) and containing 10% (v/v) defibrinated horse blood. Plates were incubated aerobically in N2/O2/C02/H2 (88:5:5:2, by vol.) in a Variable Atmosphere Incubator (VAIN; Don Whitley Scientific) or in GasPak anaerobic jars (Becton Dickinson) with a CampyPak microaerophilic gas-generating envelope and palladium catalyst, at 37 °C for 24–36 h. Clinical isolates, when used, were kindly provided by A. Clark, Department of Clinical Microbiology, Queen Elizabeth Hospital, Birmingham (strains hp2, 79, 167, 681), and by J. Bickley, PHLS, Colindale (strains A616, A654, A655, A759, A783), and were maintained as above. The latter strains were isolated in distinct geographical areas and in several different years and were minimally subcultured before use. Other organisms used were as follows: *Helicobacter mustelae* strains F831 and F931 (obtained from D. Morgan, St Bartholomew's Hospital Medical College, London) and NCTC 12198 (type strain); *Helicobacter cinaedi* strain NCTC 12423 (type strain); *Helicobacter felis* strain NCTC 11612 (type strain); *Campylobacter jejuni* strain NCTC 11828; *Campylobacter coli* strain NCTC 11366 (type strain); *V. cholerae* strain 256 (non-toxigenic) obtained from S. Knutton, University of Birmingham; and *V. parahaemolyticus* strain NCIMB 1164 (NCTC 10441) obtained from the National Collection of Industrial and Marine Bacteria. All NCTC strains were obtained from the National Collection of Type Cultures. *Campylobacter* spp. and *Helicobacter* spp. other than *H. pylori* were grown on chocolate agar at 37 °C. *V. cholerae* was grown on LB agar at 37 °C overnight; *V. parahaemolyticus* was grown on semi-solid medium containing 10 g treptone l−1, 20 g NaCl l−1 and 0.4% (w/v) Bacto Agar (Difco) at 37 °C overnight in order to produce cultures in swimmer phase with sheathed flagella, production of which was monitored by transmission electron microscopy of negatively stained bacteria.

**Electrophoresis and Western blotting.** SDS-PAGE (Laemmli, 1970) was performed in a Bio-Rad Mini-Protein II apparatus according to the manufacturer's instructions. Gels were stained with 0.01% (w/v) PAGE-blue 83 (BDH) in distilled H2O/ methanol/glacial acetic acid (10:45:45, by vol.), or proteins were transferred onto Hybond-C nitrocellulose membranes by Western blotting according to the method of Towbin et al. (1979). Blots were processed using polyclonal serum or monoclonal (culture supernatant fluid) antibodies diluted 1:500 or 1:10, respectively, in PBS (73 mM NaCl, 18 mM KH2PO4, 57 mM Na2HPO4, pH 7.4) with 0.1% (w/v) BSA and 0.1% (v/v) Tween 20 (PAT) as the first antibody. Rabbit anti-mouse immunoglobulins conjugated to horseradish peroxidase (Dako) were used as the conjugate at a dilution of 1 part in 2000 in PBS, pH 7.4. Blots were incubated for 6–18 h in both first and second antibodies. Blots were developed using 4-chloro-1-naphthol (30 mg) dissolved in 10 ml methanol, added to 40 ml PBS with 50 μl H2O2, and stopped by extensive washing in water. Molecular mass markers were from Bio-Rad, low molecular mass range.

**Two-dimensional electrophoresis (ZDGE).** A Bio-Rad Protean II 2D cell was used, in general according to the manufacturer's instructions and to O'Farrell (1975). Gels were cast in glass capillary tubes, 100 mm x 1.5 mm. For 3 ml gel solution (300–400 μg per tube) the gel contained 165 g urea, 399 μl isoelectric focusing acrylamide, 600 μl 10% (v/v) NP40 (Nonidet) and 591 μl milli-Q distilled H2O. Amphotolyte pH 5–7 (45 μl), 180 μl ampholyte pH 3–10, 3 μl 10% (w/v) ammonium persulphate and 2 μl N,N,N',N'-tetramethylethylenediamine (TEMED) were then added to the solution.

*H. pylori* (strain Roberts) (5 x 106 cells, concentration determined microscopically by Helber counting chamber) was suspended in 70 μl isoelectric focusing solubilization buffer (0.5 M Tris/Cl, pH 6.8, 2%, w/v, SDS, 5%, v/v, 2-mercaptoethanol in water), incubated for 30 min at 37 °C and then at 100 °C for 5 min, and diluted with two vols sample dilution buffer (57%, w/v, urea, 5%, v/v, 2-mercaptoethanol,
0·4%, v/v, ampholyte pH 5-7, 1·6%, v/v, ampholyte pH 3-10, 8%, v/v, 10% NP40 in water).

The sample (40 or 20 µl) was then loaded and overlaid with urea
overlay buffer (1·5 g urea, 20 µl ampholyte pH 5-7, 50 µl
ampholyte pH 3-10 made up to 5 ml with water). The gel was
focussed with 10 mM H₃PO₄ anode buffer and 20 mM NaOH
cathode buffer at 300 V for 16 h and then at 400 V for 1·5 h.

Gels were extruded from the tubes into a small amount of SDS-
PAGE reservoir buffer and loaded onto the top of a 10% (w/v)
polyacrylamide slab gel. The tube gel was loaded so that the
acidic end of the gel would run at the right-hand side of the slab
gel, nearest to the molecular mass standards. Electrophoresis
was carried out at 150 V for 4 h. Proteins separated by 2DGE
were either stained with silver by the method of Wray et al.
(1981) or were transferred to nitrocellulose membranes by
Western blotting.

Immunization of mice. Female BALB/c mice were immunized
with freshly cultured H. pylori (strain Roberts) cells that had
been washed briefly in PBS, and resuspended in adjuvant or PBS
to a density of 5 × 10⁸ cells ml⁻¹ (concentration determined by
Helber counting chamber). Mice received 0·05 ml vaccine
intrapitoneally in Freund’s complete adjuvant on day 1, the
same dose intraperitoneally in incomplete adjuvant on day 21,
and the same dose in PBS, intravenously, on day 53, followed by
fusion of splenocytes on day 56. Antiserum from mice immu-
nized thus was used to identify the flagellin band in Western
blots (Fig. 1).

Derivation of monoclonal antibody. Parent myeloma cells of
the NS1 line (Flow) were cultured and used in PEG-induced
fusion with spleen cells of immunized mice by standard
procedures (Harlow & Lane, 1988).

Selection of reactive clones. Between 10 and 14 d after fusion,
wells containing hybridoma colonies of 0·5–1 mm diameter
were sampled by removal of 100–200 µl supernatant fluid. This
was diluted 1:10 and reacted with strips of nitrocellulose blotted
with SDS-PAGE-separated whole-cell proteins of strain
Roberts.

Immunogold labelling. All reactions were performed at room
temperature. Formvar-coated grids bearing specimens were
blocked in 3% (w/v) BSA for 1 h, then floated, inverted on
50 µl antiserum diluted 1 part in 1000 in PBS containing 0·1%
(w/v) BSA and 0·1% (v/v) PAT, and washed by floating for
3 min on 5×50 µl drops PBS. The grids were then similarly
exposed to goat anti-mouse immunoglobins conjugated to
10 nm colloidal gold (BioCell, Cardiff) which was diluted 1:10
in PBS, washed in PBS followed by distilled water, negatively
stained with 5% (w/v) ammonium molybdate solution, air
dried and then examined by transmission electron microscopy
using a Philips EM301 or JEOL 1200 EX instrument operated
at 80 kV.

Determination of isotype of murine monoclonal antibody
GF6. The immunoglobulin isotype of the monoclonal
monoclonal antibody GF6 was determined using a Murine Immunoglobulin
Isotyping Kit (Sigma).

Reactivity of monoclonal antibody GF6 with semi-purified H.
pylori urease. Semi-purified, lyophilized urease from H. pylori
strain 11637, prepared by preparative isoelectric focusing
(Rotorfor, Bio-Rad) of an aqueous sonicate of
H. pylori
was kindly donated by Dr A. Cockayne, University of Nottingham.
The enzyme and whole-cell samples of H. pylori strains 11637
and Roberts were subjected to SDS-PAGE and the separated
proteins then transferred to nitrocellulose membranes by
Western blotting. The blot was probed with monoclonal
antibody GF6, at a dilution of 1:10 in PAT, and incubated with
rabbit anti-mouse immunoglobulin-HRP conjugate as above.
After development and photography, the blot was washed in
several changes of PBS and re-processed with a rabbit polyclonal
antiserum (kindly donated by Dr Chris Clayton, Glaxo, UK)
raised against recombinant urease of H. pylori expressed in
Escherichia coli (diluted 1:100 in PAT), followed by goat anti-
rabbit immunoglobulins conjugated to HRP (ICN-Flow, UK)
which had been diluted 1:2000 in PBS, pH 7-4, and blots
processed as described earlier.

2DGE of H. pylori whole cells and immunoblotting with
monoclonal antibody GF6 and with the anti-urease polyc-
onal serum. H. pylori whole-cell proteins were separated by
2DGE as above. Gels loaded with 20 µl of sample were stained
with silver following electrophoresis. Gels loaded with 40 µl of
sample were immunoblotted with either monoclonal antibody
GF6 at a dilution of 1 part in 10 in PAT, or rabbit anti-urease
polycanal serum at a dilution of 1 part in 100 in PAT (replica
blots were used), and blots processed as described above.

RESULTS

Reactivity of monoclonal antibody GF6 by Western
blot

Monoclonal antibody GF6 (isotype IgG2b) was selected for
its immunoblot reactivity with an antigen band in
strain Roberts of molecular mass 29 kDa (Fig. 1).

Fig. 1. SDS-PAGE and immunoblot analysis of polypeptides of
H. pylori, strain NCTC 11637. Protein profiles are shown in
tracks A and B. The gel was stained with Coomassie blue in
track A and the Western blot with Ponceau red in track B. Track
C shows development of an immunoblot with a polyclonal
antibody containing mainly antibody to the 54 kDa flagellin
(the most strongly stained band in this track), but also to
other proteins including the 30 kDa UreA protein, which is level
with the 31 kDa marker. Track D is an immunoblot developed
with monoclonal antibody GF6, showing only a band at about
29 kDa, clearly distinct from the 30 kDa UreA protein. The
positions of molecular mass markers (kDa) are shown to the
left.
Comparison of immunoblot and protein profiles showed that the reactive band was very close to a major stained component believed to correspond to the smaller subunit of the urease enzyme, UreA, but it nevertheless appeared distinct from UreA.

Reactivity of monoclonal antibody GF6 with SDS-PAGE-separated whole-cell proteins of a variety of other strains of *H. pylori* in Western blots (Fig. 2) showed that in all cases a band of approximately 29 kDa reacted strongly with the antibody. None of the strains tested failed to react with this antibody. There was some evidence of heterogeneity between strains, e.g. strain A783 (track 9 of Fig. 2), in the exact molecular mass and abundance of the antigen.

Immunoblots were also performed against whole-cell proteins of other species of *Helicobacter*, and of representative *Campylobacter* and *Vibrio* species (Fig. 3). The only reactions seen were weakly stained bands in *V. cholerae* and *V. parahaemolyticus*, at a molecular mass of about 65 kDa, a considerably higher molecular mass than in *H. pylori*.

**Immunogold labelling of *H. pylori* by monoclonal antibody GF6**

In order to attempt to localize the GF6-reactive antigen, and to check for its surface exposure, immunogold labelling was employed. The labelling obtained showed that the antigen was localized to the sheath structure of sheathed flagella (Fig. 4a). Flagella which had lost their sheaths were not immunogold labelled. In general, the outer membrane of intact cells was also free of immunogold label (Fig. 4b), although some cells showed patches of label associated with the cell surface where it appeared that debris, probably including flagellar sheath material which had become detached from the flagella, was associated with the cell.

**Differentiation of the GF6-reactive antigen from UreA**

In view of the apparent close similarity in molecular mass of the GF6-reactive antigen to that of UreA, it was important to establish unequivocally that UreA was not the reactive antigen. Two approaches were adopted. Firstly, a semi-purified preparation of *H. pylori* urease
Flagellar sheath protein of *Helicobacter pylori*

Fig. 4. Transmission electron micrographs of immunogold labelling of flagellar sheaths of *H. pylori* strain Roberts by monoclonal antibody GF6. Note the heavy immunogold labelling of the flagellar sheath in contrast to the absence of label on unsheathed flagella in (a), and the absence of label on the cell surface in (b). Bars represent 0.2 μm.

from strain NCTC 11637, and a polyclonal rabbit antiserum specific for recombinant urease of *H. pylori* expressed in *E. coli*, were compared by immunoblot with the monoclonal antibody GF6-reactive antigen and with monoclonal antibody GF6. Initially, whole-cell antigens of *H. pylori* of both the Roberts and NCTC 11637 strains as well as the urease preparation were blotted and reacted with monoclonal antibody GF6. Only the whole-cell-
Fig. 5. Western blot analysis of the GF6-reactive polypeptide and urease polypeptides of strains Roberts and NCTC 11637. The same set of four tracks is shown to the left (marked 1-4, reacted with monoclonal antibody GF6 only) and to the right (marked 5-8, the same blot re-reacted with polyclonal antisera to urease). Tracks marked 1, 3, 5 and 7 contained whole-cell proteins (tracks 1 and 5, Roberts strain; tracks 3 and 7, strain NCTC 11637), while for comparison tracks marked 2, 4, 6 and 8 contained semi-purified urease of strain NCTC 11637. The molecular masses (kDa) of immunoreactive bands are shown to the right.

derived antigens reacted strongly (Fig. 5, tracks 1–4). The blots were then re-incubated with anti-urease serum and goat anti-rabbit immunoglobulins conjugate to reveal strong bands in all preparations which were at the positions of the large and small subunits of the urease enzyme (Fig. 5, tracks 5–8). It appeared that the monoclonal antibody GF6-reactive bands were slightly thickened towards the top of the blot after the second incubation, and that the centre of the small urease subunit band was slightly above that of the combined antigens in the whole-cell preparations. Examination of Fig. 1 also indicates quite clearly the separation of the monoclonal antibody GF6-reactive band from the more prominent 30 kDa major stained and polyclonal antibody-reactive band attributed to the UreA protein.

Secondly, 2DGE was used to attempt separation of the UreA and GF6-reactive proteins. Fig. 6(A) shows the pattern of protein spots obtained after silver staining of a 2DGE gel, with the monoclonal antibody GF6-reactive protein, identified as explained below, arrowed. In Fig. 6(B), a blot of a replicate gel was reacted with monoclonal antibody GF6. A strongly reactive streak was visible at the expected molecular mass position in the second dimension, across the basic half of the blot. Comparison with the positions of spots in Fig. 6(A) shows that this streak is more basic than the majority of major proteins. Another replicate was reacted with the anti-urease serum used in Fig. 5 (Fig. 6C); this showed reactivity of the larger urease subunit UreB, but no reactivity at the

Fig. 6. Two-dimensional gel electrophoretic and immunoblot analysis of proteins of H. pylori, strain Roberts. In panels A and C, the second dimension SDS-PAGE gels included tracks at the right-hand edge which contained whole-cell proteins, as well as molecular mass markers in panel A. Panel A shows the pattern of silver-stained polypeptides obtained, with positions of molecular mass marker proteins (kDa) shown to the right; panel B is a two-dimensional immunoblot processed with monoclonal antibody GF6, showing a streaked immunoreactive spot at approximately 29 kDa (arrow) – the corresponding spot on the stained pattern is also arrowed in panel A; panel C is a replicate blot processed with polyclonal anti-urease antibody, showing both one-dimension bands obtained from the subunits UreA (30 kDa) and UreB (66 kDa) as indicated to the right, and a clear two-dimensional spot for the UreB subunit (bold arrow); there is no corresponding spot for the UreA protein. In isoelectric focusing, the anode was to the right and the cathode to the left.
molecular mass position expected for UreA, despite reactivity at both positions in a single-dimension track of whole-cell proteins at the edge of the blot.

DISCUSSION

The monoclonal antibody GF6 appears to react with a novel flagellar sheath polypeptide of *H. pylori* which is present in all strains examined. There does not appear to be a polypeptide of similar molecular mass and bearing the same antigenic epitope among the closely related bacterial species examined, although the presence of an antigenically related polypeptide of higher molecular mass in *Vibrio* species suggests the possibility of a functionally related domain in a protein which might have a related function in these organisms.

An important feature of the data described here is the evidence that the putative flagellar sheath protein is not identical to the small urease subunit UreA. The electrophoretic data presented appear unequivocal, both in one-dimensional and two-dimensional formats. Staining of one-dimensional gels suggests that the sheath protein has a slightly lower molecular mass than UreA, and also, based on Coomassie blue and Ponceau red staining, that the sheath protein is significantly less abundant than UreA. In two-dimensional analysis, UreA was lost from the gel, presumably due to its predicted high isoelectric point, which we have calculated from the published DNA sequence (Clayton *et al.*, 1989) to be 9.4. Also potentially identical to the protein we describe here is a protein of 30 kDa, believed to be a porin, reported by Tufano *et al.* (1994). In the absence of any indication of the location of this 30 kDa protein on the cell surface, we cannot infer whether it is identical or not to the monoclonal antibody GF6-reactive protein we describe.

The identification of a flagellar sheath protein is potentially important in advancing our knowledge of the structure and function of bacterial flagellar sheaths. To date most hard information about the nature of the sheath has rested solely on morphological observation, suggestive only of a membrane-like structure, most elegantly demonstrated in *H. pylori* by Geis *et al.* (1993). Those few analyses that have been performed have suggested the presence of lipopolysaccharide (LPS) in the flagellar sheath of *V. cholerae* (Fuerst & Perry, 1988) and of LPS and lipids in *B. bacteriovorus* (Thomasow & Rittenberg, 1985). Proteins have also been demonstrated in flagellar sheaths of *V. cholerae* (Fuerst & Perry, 1988; Hranitzky *et al.*, 1980). The flagellar sheath, thus, might simply represent an extension of the outer membrane, with a function no different from the latter. In the case at least of *H. pylori*, this now seems unlikely since the protein reported here appears uniquely associated with the sheath. So far nothing is known of structure or other properties of this protein other than that it has a quite strongly basic isoelectric point.

Possible functions of the flagellar sheath and/or the constituent protein include a role in flagellum-mediated adhesion to surfaces, such as gastric epithelial cells. Studies with gastric carcinoma cell line ATCC CRL 1739 showed that, while organisms (Roberts strain) were strongly adhesive to these cells and adhesion could be blocked by incubation with homologous polyclonal rabbit antibody to whole organisms, no such blocking activity was observed with monoclonal antibody GF6 (C. J. Luke, unpublished observations). Another possible function of the sheath is in a protective role for the flagellum, which might be shielded from dissociation of subunits from one another by stomach acid, for example. A sheath protein might also interact structurally with the flagellar shaft proper to mediate cohesion of the sheath to the flagellum. Until the protein is more thoroughly characterized, for example by gene cloning and sequence analysis, these questions must remain unanswered.

The flagellar sheath protein of *H. pylori* has the potential to be exploited as an antigen quite specific to this organism, since it is present in all strains so far tested and does not appear to be present in closely related *Helicobacter* species or in other gastro-intestinal bacterial species so far examined.

ACKNOWLEDGEMENTS

We thank the Wellcome Trust for financial support, David J. Reynolds for advice on two-dimensional electrophoresis, and Stephen Ward for expert assistance.

REFERENCES


Received 5 August 1994; revised 13 October 1994; accepted 17 November 1994.