Variation of the O antigen of *Campylobacter jejuni* in vivo

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Summary. During the course of a clinical study on patients with campylobacteriosis, three consecutive isolates of *Campylobacter jejuni* from the same patient were sent for O-serotyping. Marked differences in the specificities of the O antigens of the isolates were observed between the first and third isolates when a passive haemagglutination assay system developed for serotyping *C. jejuni* was used. Differences in specificity were also demonstrated by immunoblots of lipopolysaccharides (LPS) from proteinase K-digested whole cells. The three isolates could not be distinguished either by restriction endonuclease analysis of chromosomal DNA, by gel electrophoresis of whole-cell proteins or by silver-stained LPS gels, thus providing evidence that they were of the same strain and that antigenic variation had occurred *in vivo*.

Introduction

The pathogenic organism *Campylobacter jejuni* can be serotyped on the basis of its O antigens with the use of a thermostable antigen extract and a passive haemagglutination assay.^{1,2} Although generally there are very few cross-reactions among the 42 serotypes, there is an important group comprising the O:4, O:13, O:16, O:43 and O:50 serotypes that do cross-react and seem to be closely related.^{2,3} Clinical isolates have been found with similar cross-reactions and epidemiologically linked isolates from outbreaks have been shown to differ in their reaction to the antisera against these five serotypes.^{3–5} This suggests the possibility of a host effect on the expression of LPS antigens. It may be an important phenomenon to take into account in epidemiological investigations, as a number of outbreaks have been the result of strains from this antigenic group.

Materials and methods

Bacterial strains

Three isolates of *C. jejuni* were obtained from the same diarrhoeic patient. *C. jejuni* A1613 was the first stool isolate, A1614 the second and A1615 the third. The isolates were subcultured on Columbia blood agar in a micro-aerophilic environment (O₂ 5% v/v, CO₂ 10% v/v) at 37°C for 24 h and growth from single colonies was frozen at –70°C in protease peptone 10% w/v containing glycerol 20% v/v. From this stock, plate cultures were produced to provide the biomass for preparation of LPS and proteins.

Serotyping

The five antisera used in this study were serotyping antisera prepared in rabbits by intravenous injection of bacterial saline suspensions of the *C. jejuni* serotypes MK7(O:4), MK16(O:13), TGH4936(O:16), VanA5(O:43) and MK307(O:50) as described previously.^{1,2} The passive haemagglutination assay (PHA) was used to titrate the antisera.^{1,2} To serotype the organisms, a heated cell extract was prepared by harvesting confluent growth from three plates into 3 ml of saline (NaCl 0.85% w/v), heating (100°C, 1 h), and centrifugation (8000 g, 10 min). For titration of antisera, the supernate was diluted 1 in 10 in IPBS (isotonic phosphate-buffered saline) and mixed with an equal volume of a washed suspension of sheep erythrocytes (1% v/v). After sensitisation for 1 h at 37°C, erythrocytes were washed three times in IPBS and resuspended to the original volume. Antisera (25 µl) were diluted two-fold in microdilution plates (U-shaped wells), an equal volume (25 µl) of sensitised erythrocyte suspension was added to each well and the plates were incubated at 37°C for 1 h and then overnight at 4°C. The titre of the antiserum was taken as the highest dilution showing agglutination of erythrocytes.

Protein and LPS preparations

Whole-cell protein preparations were made by removing growth from a single plate with 5 ml of IPBS and diluting the cells to an optical density of 0.3 at 600 nm. A 1.5-ml volume was transferred to an eppendorf tube, the cells were centrifuged and the pellet was resuspended in 200 µl of lysis buffer (glycerol 20% v/v, mercaptoethanol 5% v/v, sodium dodecyl sulphate 4.6% w/v, bromophenol blue 0.004% w/v, 0.625 M Tris-HCl, pH 6.85). To ensure efficient lysis,

tubes were heated at 100°C for 10 min and cooled on ice. All mol. wt markers were obtained from BioRad Laboratories, Richmond CA, and included for the low-mol. wt standards, phosphorylase B (97 400), bovine serum albumin (66 200), ovalbumin (45 000), carbonic anhydrase (31 000), soybean trypsin inhibitor (21 500) and lysozyme (14 400). In addition to the first three proteins, the high mol. wt markers included myosin (200 000) and β -galactosidase (116 250).

LPS preparations were made by proteinase K digestion of the washed whole cells.⁶ Lysis buffer (40 μ l) containing proteinase K 2.5 mg/ml was added to each tube and incubated overnight at 37°C and then for 2 h at 65°C.

Gel electrophoresis and immunoblotting

Whole-cell protein preparations (30 μ l) and proteinase K-digested whole cells (30 μ l) were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with the discontinuous buffer system of Laemmli.⁷ Electrophoresis was at a constant current of 35 mA with a 5% stacking and 10% separating gel. Protein gels were stained with Coomassie Brilliant Blue and LPS gels were stained with silver.⁸

After electrophoresis, gels were transferred to nitrocellulose by the Trans-Blot system (BioRad) for 2.5 h at 65 V.⁹ Immunoblots were performed as described previously³ with rabbit antisera diluted 1 in 50.

DNA electrophoresis

Total cellular DNA was prepared according to techniques published previously.¹⁰ Approximately 20 μ g of DNA from each isolate was digested with an excess of *Bam*HI, *Pvu*II, *Hind*III or *Hae*III for 4 h at 37°C according to the manufacturer's directions (Boehringer-Mannheim). DNA fragments were separated by electrophoresis in an agarose (0.7% w/v) gel at 30 V and visualised by ethidium bromide (1 μ g/ml) staining.

Results

The results obtained from serotyping the three clinical isolates A1613, A1614 and A1615 are shown in the table. The first stool isolate belonged to serogroup O:4,13 but had a minor cross-reaction with antiserum to group O:50 (table). The second serotyped as O:4,13, very similar to the first isolate but with an additional minor cross-reaction with O:16 serum. However, the third isolate had a strong reaction only with antiserum O:13, no reaction with either the O:4 or O:16 antisera, and, like the other two, a minor cross-reaction with the O:50 antiserum.

Because the PHA test is primarily based on the detection of IgM antibodies and is thought to have a

Table. Results of the passive haemagglutination assay with the antigenic variants of *C. jejuni*

Strain	Titre with serotyping antisera*				
	O:4	O:13	O:16	O:43	O:50
A1613	320	2560	<40	<40	40
A1614	640	2560	40	<40	40
A1615	<40	5120	<40	<40	40

* All titres are expressed as the highest dilution of the antiserum showing haemagglutination. The lowest dilution used was 40.

different level of sensitivity to our immunoblotting system which detects exclusively IgG antibodies, absolute correlation of results is not possible. However, when the antisera used to obtained PHA titres were used in immunoblots with LPS from the three clinical isolates, the results were comparable, i.e., LPS from each isolate reacted with the O:13 antiserum but only LPS from isolates A1613 and A1614 reacted weakly with the O:4 antiserum.

To determine if the three isolates were the same strain which had undergone antigenic variation, a number of tests were performed. Coomassie Brilliant Blue-stained whole-cell protein profiles showed that for the *c.* 30–50 proteins present, all three strains were identical (fig. 1) but distinct from the serostrains for serogroups O:4, O:13, O:43 and O:50. Low-mol. wt LPS material from *C. jejuni* is characteristically observed in silver-stained gels of proteinase K-digested whole cells. The virtually identical profiles for LPS from isolates A1613, A1614 and A1615 (fig. 2) also suggested that the three were of the same strain. Moreover, the LPS patterns of the three isolates were

Fig. 1. Whole-cell protein profiles of the clinical isolates of *C. jejuni*. Lane 1, low-mol. wt markers; 2, *C. jejuni* strain A1613; 3, strain A1614; 4, strain A1615; 5, high-mol. wt markers.

Fig. 2. Silver-stained gel of proteinase K-digested whole cells showing the LPS profiles of the clinical isolates of *C. jejuni*. Lane 1, *C. jejuni* A1613; 2, strain A1614; 3, strain A1615.

readily distinguishable from the LPS patterns previously described³ for serostrains O:4, O:13, O:16, O:43 and O:50.

Total cellular DNA was extracted from the three isolates and from the serostrains for O groups 4, 13, 16, 43 and 50. When undigested DNA was electrophoresed, the only strain that showed the presence of plasmid DNA was the serostrain for *C. jejuni* O:16, confirming an observation reported previously.⁴ The DNA preparations were subjected to restriction endonuclease analysis (REA) with four different enzymes. *Bam*H1 produced restriction patterns for each of the five serostrains that were virtually identical and so were not considered useful for comparisons. The three other enzymes (*Hae*III, *Hind*III and *Pvu*II) produced patterns for the O:43 and O:50 strains that were markedly different from each other and from the patterns produced for the O:4, O:13 and O:16 strains. This is consistent with findings reported earlier with the use of *Hind*III only.⁴ However, DNA from *C. jejuni* serostrains O:4 and O:13 gave restriction patterns with the enzymes *Pvu*II and *Hind*III that were identical. The use of *Hae*III, gave very similar patterns that differed in one or two bands (fig. 3, lanes 4 and 5), confirming earlier results that had shown

these two strains to be nearly identical.⁴ Although the presence of plasmid DNA in serostrain O:16 interfered with a comparison of banding patterns produced with *Hind*III and *Pvu*II, it was found that *Hae*III did not cut the plasmid (fig. 3, lane 3). Moreover, this enzyme produced a restriction pattern for serostrain O:16 by which it could be easily distinguished from patterns produced for serostrains O:4 and O:13, although all three strains had many bands in common (fig. 3, lanes 3–5). These results suggested that the enzyme *Hae*III had the greatest ability to differentiate between these strains, *Bam*H1 had the least ability, whereas *Pvu*II and *Hind*III were somewhere between the two.

In examining the three isolates it was found that although each of the four enzymes produced restriction patterns with many bands of <0.5–40 kb, differentiation of the isolates could not be made on the basis of these patterns even when *Hae*III was used (fig. 3, lanes 6–8). Regardless of which enzyme was used, the fragment profile produced for the isolates could be clearly distinguished from profiles of the serostrains. However, there were many bands in common between the clinical isolates and serostrains O:4, O:13 and O:16 when *Hae*III was used. Two fragments enabled distinction between the *Hae*III restriction pattern characteristic of the clinical isolates and the patterns of serostrains O:4, O:13 and O:16 suggesting that the isolates constitute a separate strain that is closely related to these three *C. jejuni* serostrains (fig. 3).

Discussion

The results obtained in this study provide evidence that a strain of *C. jejuni* is able to shift the antigenic makeup of its cell surface by exposing new determinants or by losing others. Although isolates have been obtained from patients within an outbreak that expressed antigenic specificities of different cross-reacting groups^{3,4} this, to our knowledge, is the first report of a shift in antigenic spectrum within one host. The virtual identity of results for the three clinical isolates from the same patient obtained by REA with four endonucleases indicated that the observed antigenic variation had occurred during the course of an infection without major rearrangements in genetic components. Therefore, it is hypothesised that the variation is probably a result of altered gene expression, a phenomenon known to occur among the Enterobacteriaceae.^{11,12} O-antigenic variation has also been observed in a closely-related species, *C. coli*, when organisms were maintained for prolonged periods in the laboratory in static culture; but in that case, minor changes in the restriction patterns were noted.¹³ It is interesting that the *C. coli* strain had gained a determinant that was detected by an antiserum specific for a strain of *C. jejuni*. Antigenic variation has been shown to occur among other cell-surface antigens of *Campylobacter* species. It has been

Fig. 3. Ethidium bromide-stained agarose gel of *Hae*III-digested chromosomal DNA of various strains of *C. jejuni*. Lane 1, serostrain O:50; 2, O:43; 3, O:16; 4, O:13; 5, O:4; 6, strain A1613; 7, A1614; 8, A1615. The plasmid band in serostrain O:16 is indicated (*); differences in fragments between O:4, O:13 and O:16 are indicated by arrow heads.

demonstrated for the flagellar protein of *C. coli*¹⁴ and the surface-array protein of *C. fetus*.^{15,16}

An ability to perform such shifts within a host as we have shown here would be expected to have an important role in determining virulence of these organisms. Furthermore, the occurrence of the phenomenon should be taken into account when serotyp-

ing data are interpreted in epidemiological studies of outbreaks with strains bearing O specificities detected with the serogroup O:4, O:13, O:16, O:43 and O:50 antisera.

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