Hepatotoxic activity of *Campylobacter jejuni*

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Summary. Hepatotoxic factor(s) were isolated from whole-cell lysates of *Campylobacter jejuni* GIFU 8734 and purified by chromatography. A single intravenous injection of 10 µg of this factor reproducibly produced hepatitis in mice, as determined by histology and liver function tests. The hepatic lesions were very similar to those evoked by *C. jejuni* infection. Tissue-culture studies with mouse hepatocytes demonstrated that low concentrations of the factor caused release of hepatic enzymes into the medium without appreciable cytolysis. High concentrations of the factor induced cytolysis. These effects were neutralised by antiserum to the factor, but not by antisera to the lipopolysaccharide of *C. jejuni* or to the heat-labile enterotoxin of *Escherichia coli*. Among 20 clinical isolates of *C. jejuni*, only four evoked hepatitis in mice and produced the hepatotoxic factor.

Introduction

Campylobacter jejuni, a gram-negative spiral bacterium, is a major cause of diarrhoea in man.^{1,2} Enterotoxigenicity is recognised as one of the contributory factors to its virulence.^{3–5} Various clinical and animal studies indicate that the organism can also cause invasive disease.^{6–8} Hepatic involvement, as well as intestinal lesions, has been associated with campylobacter infection.^{9–11} Previously, we have reported that mice developed focal infiltrative necrotic lesions in the liver within 1 or 2 months of oral infection with *C. jejuni*.¹² The hepatic damage was demonstrated by histology and liver function tests. However, the mechanism by which *C. jejuni* induces hepatitis in mice has not been established.

In the present study, we examined the production of a hepatotoxic factor by clinical isolates of *C. jejuni*.

Materials and methods

Bacterial strains

C. jejuni GIFU 8734¹² was used as a reference strain. Strains Nara-3, -5, -6, -24, -42, -68, -69, -73, -77 and -79, KZ-4, -6, -9, -12 and -14, MT-6 and -10 and DK-1 were clinical isolates from adult patients with campylobacter enteritis. Strains MT-7 and -9 were isolated from asymptomatic carriers. Bacteria were grown routinely at 42°C for 24 h on Brucella Agar (Difco) containing horse blood 10% v/v in an anaerobic jar with a Gas-Pak (BBL

Microbiology Systems) but without catalyst. The bacteria grown on these plates were transferred into the liquid medium described previously¹² and incubated at 42°C for 48 h in a micro-aerobic atmosphere.

Preparation of the hepatotoxic factor(s)

Cells cultured in the liquid medium were harvested and washed twice with 10 mM Tris-HCl buffer, pH 7.2. The pellets were resuspended in the same buffer containing 10 mM MgCl₂, DNAase type I (Sigma) 1 mg/L and sodium dodecyl sulphate (SDS) 0.05% w/v, then disrupted by ultrasonic oscillation (Ultrasonic Oscillator, Kaijyod-enki, Tokyo, Japan) for 20 min at a plate current of 300 mA. The sonicates were centrifuged at 330g for 20 min and the supernates were re-centrifuged at 4200g for 60 min. The supernates thus obtained were centrifuged at 12 000g for 60 min, and then at 90 000g for 90 min. The final pellet was washed twice with distilled water and lyophilised after dialysis against distilled water. The lyophilised material was dissolved in 10 mM sodium phosphate buffer, pH 7.2 (PB), and solid ammonium sulphate was added to 30% saturation. The mixture was centrifuged at 13 000g for 30 min and the pellet, designated P30, was re-dissolved in PB. To the supernate, solid ammonium sulphate was added to 65% saturation before re-centrifugation at 13 000g for 30 min. The pellet, designated P65, was suspended in PB. Both P30 and P65 pellets were dialysed for 72 h at 4°C against several changes of distilled water and then lyophilised.

Chromatographic separation of P65

The lyophilised P65 material was re-dissolved in PB containing 0.05 M NaCl and applied to a column (1.6 × 40 cm) of Sepharose 4B (Pharmacia Fine Chemicals,

Uppsala, Sweden) which had been pre-equilibrated with the same buffer. Fractions (5 ml) were collected at a flow rate of 10 ml/h. Those fractions that exhibited cytotoxicity against hepatocytes were pooled and applied to a further column (2.0 × 50 cm) packed with DEAE-Sepharose CL-6B (Pharmacia) which had been pre-equilibrated with PB containing 0.05 M NaCl. After the sample was applied, the column was washed with two column-volumes of the same buffer before elution with 400 ml of PB containing a gradient (0.05–0.8 M) of NaCl. Fractions (5 ml) were collected at a flow rate of 20 ml/h. Those with hepatotoxic activity were pooled and dialysed overnight at 4°C against 100 volumes of Hanks's Balanced Salts Solution (HBSS) without phenol red and then, under identical conditions, against 100 volumes of Williams E Medium (Flow Laboratories, McLean, VA, USA) supplemented with NaHCO₃ 0.07% w/v, 20 mM HEPES, 2 mM L-glutamine, penicillin, 60 mg/L, streptomycin 50 mg/L and gentamicin 5 mg/L.

Hepatocyte cultures

Hepatocytes were isolated from anaesthetised mice by the method of Berry and Friend.¹³ The liver was initially perfused with 50 ml of Ca²⁺- and Mg²⁺-free Hanks's solution (CMFS), then with 100 ml of CMFS containing collagenase (type IV, Sigma) 0.05% w/v, bovine serum albumin (BSA, fraction V, Sigma) 0.1% w/v and 4 mM CaCl. All perfusion media were warmed to 37°C and gassed with a mixture of O₂ 95% and CO₂ 5% for at least 30 min before use. After the second perfusion, the liver was removed from the animals, rinsed with cold CMFS and placed in a beaker containing 50 ml of cold CMFS. The loose parenchyma was combed free of supporting fibrous tissue, and the cells were dispersed with a spatula. The cell suspensions were filtered through gauze, centrifuged at 50g for 5 min and resuspended in cold HBSS containing fetal calf serum (FCS) 5% v/v. The cells were then washed twice with the same solution and finally resuspended in HBSS containing FCS. The number of cells was determined with a haemocytometer and cell viability was determined by the exclusion of trypan blue. Only those preparations containing more than 95% viable cells were used in tissue culture.

Hepatocytes were suspended to a density of 2 × 10⁶ cells/ml in Williams E medium supplemented with 10⁻⁷ M insulin, 10⁻⁸ M dexamethasone phosphate and FCS 5% v/v, and 200-μl amounts of these suspensions were seeded into flat-bottomed wells in tissue culture plates (Falcon 3047, Nippon Becton Dickinson Co. Ltd, Tokyo, Japan) which had been coated with collagen (type I, Nitta Gelatine Co., Osaka, Japan) and to which 300 μl of the medium was added. After incubation for 2 h at 37°C in CO₂ 5%, unattached cells were removed and fresh medium (1 ml) was added. After 24 h, the culture medium was discarded and fresh medium (500 μl) was added. Experiments were started after a further 24-h culture period. At this stage, the culture medium was discarded and fresh medium (450 μl) was added to each well. Thereafter, 50 μl of HBSS (control sample) or of

serial dilutions of the test material were added to the wells and the plates were incubated at 37°C in CO₂ 5%.

Determination of viable cell number

After a 24-h culture period, the hepatocyte monolayers were washed twice with CMFS and the cells were detached by adding a solution of trypsin 0.1% w/v with EDTA 0.02% w/v in CMFS. The cell suspensions obtained were mixed with an equal volume of fresh medium containing FCS 5% v/v. The cells were washed twice and suspended in CMFS. The number of viable cells was determined by measurement of the exclusion of trypan blue in a haemocytometer.

Quantification of albumin and excreted enzymes

The amount of mouse albumin secreted into the medium was measured by rocket immuno-electrophoresis.¹⁴ Briefly, antiserum (100 μl) to mouse serum albumin (Organo Teknika Corp. -Cappel Products, Westchester, PA, USA) was mixed at 50°C with 10 ml of agarose 1% w/v in Tris-barbital buffer pH 8.6.¹⁴ Pooled mouse serum albumin (fraction V) was diluted in culture medium and used as a standard. Medium from freshly isolated hepatocytes was included in each gel to serve as a positive control.

Glutamic-oxaloacetic transaminase (EC 2.6.1.1.: GOT), and lactic dehydrogenase (EC 1.1.1.27: LDH) activities were measured in the supernates. GOT was measured by the method of Henley and Pollard¹⁵ and LDH by the method of Wróblewski and LaDue.¹⁶ The reactions were started by adding 100 μl of the supernate to 1.9 ml of reaction mixture and the change in absorbance at 340 nm was measured at 25°C in a spectrophotometer. Each enzyme activity was expressed in milli-units (mU)/ml of supernate; 1 mU was the amount of enzyme that converted 1 M nmol of substrate/min.

The enzyme activities in the sera of mice were measured with a Hitachi 706 D autoanalyser, as described previously.¹²

Histological studies

Multiple sections of the liver were examined after the tissues had been embedded in paraffin, sectioned and stained with haematoxylin and eosin by the conventional method. All slides were read by two observers, neither of whom knew the origin of the individual samples.

Neutralisation test

Lipopolysaccharide (LPS) was extracted from *C. jejuni* GIFU 8734 by the hot phenol-water technique¹⁷ and purified by the method of Logan and Trust.¹⁸ Heat-labile enterotoxin (LT) was extracted and purified from a strain of *Escherichia coli* (kindly provided by Dr M. Ehara) by the method of Clements and Finkelstein.¹⁹ Antiserum to

the LPS of *C. jejuni* was prepared in ddY mice by the method reported previously.²⁰ Antiserum to the LT of *E. coli* was also prepared in mice by the method of Svennerholm *et al.*²¹

To prepare antiserum to the hepatotoxic factor(s), one volume of the sample (20 mg/L) was treated with an equal volume of aqueous formaldehyde 6% v/v at 4°C for 3 days and then dialysed against 1000 volumes of PB for 3 days with repeated changes of buffer. After membrane filtration, the treated sample (10 mg/L in a volume of 500 µl) was emulsified in an equal volume of Freund's incomplete adjuvant. Mice were immunised by subcutaneous injection of 50 µl of the material at the base of the tail, four times at 12-day intervals, and antisera were extracted from groups of 10 mice 7 days after the final injection.

In neutralisation tests, 50 µl of hepatotoxic factor(s) (20 mg/L) was mixed with an equal volume of serially-diluted antiserum and incubated at 37°C for 30 min; 50-µl amounts of these mixtures were then added to hepatocyte cultures. Hepatotoxic factor(s) treated with normal mouse serum was used as a positive control. The GOT activity of each undiluted antiserum was measured before use. After 24 h, cytotoxicity to hepatocytes was measured by exclusion of trypan blue, and leakage of GOT into the supernate was measured as described above.

Infection of mice

To determine the ability of clinical isolates to colonise the murine liver, groups of five mice were infected by

introducing 0.1 ml of HBSS containing 10⁶ cfu directly and atraumatically into the stomach. The mice were killed 30 days after infection and their livers were removed aseptically. The numbers of *C. jejuni* cells in the liver and intestine were measured as described previously.¹²

Statistical tests

Student's *t*-test was used for statistical comparisons.

Results

Hepatotoxicity of P65 fractions from *C. jejuni*

The hepatotoxicity of the P65 fractions prepared from *C. jejuni* GIFU 8734 and *E. coli* 271-6 were compared. The P30 fractions from both species lacked hepatotoxicity. An intravenous injection of ≥ 50 µg of the P65 fraction from *C. jejuni* increased serum concentrations of GOT in mice 5 days after injection. Leakage of GOT increased in a dose-dependent manner in the dose range 50–200 µg of P65 fraction/mouse (fig. 1). In contrast, the P65 fraction from *E. coli* did not increase GOT concentrations, even when doses as high as 200 µg/mouse were administered.

Histological study demonstrated that the P65 fraction from *C. jejuni* affected the parenchymal cells and induced patchy necrotic changes by 7 days

Fig. 1. Liver function in mice given the P65 fraction from *C. jejuni* GIFU 8734 (□) or *E. coli* 271-6 (■) by intravenous injection. Groups of five mice received the doses of P65 material indicated and were bled 5 days after injection. The data were obtained from two separate experiments and are expressed as the mean ($\pm$ SD) for the 10 mice.

Fig. 2. Histological changes in the liver: (a) 7 days after injection of P65 material from *C. jejuni* GIFU 8734, 50 µg mouse; (b) 48 h after injection of peak C material, 10 µg; and (c) 20 days after injection of peak C material, 10 µg. Bar = 100 µm.

after injection (fig. 2a). The P65 fraction of *C. jejuni* also affected cell membranes of hepatocytes as determined by exclusion of trypan blue by cells exposed to doses >50 mg/L. Its cytotoxic effect increased linearly in the dose range 50–100 mg/L. It was toxic for 58% of cells at a concentration of 100 mg/L, whereas only 15% cytotoxicity was obtained with the P65 fraction from *E. coli* at 100 mg/L (fig. 3). In the dose range 10–100 mg/L, the P65 fraction from *C. jejuni* enhanced leakage of GOT and LDH, whereas albumin synthesis was reduced by only 20% at a dose of 100 mg/L (table I). In contrast, the P65 fraction of *E. coli* induced a slight elevation of GOT and LDH activities in the culture supernates without significant reduction of albumin synthesis.

Chromatographic separation of hepatotoxic factor(s)

Three peaks were obtained by chromatography of the P65 fraction from *C. jejuni* on Sepharose 4B (fig. 4a). Hepatotoxicity, as determined by the dye exclusion test, was associated with the second peak. The fractions containing this peak were pooled and subjected to chromatography on DEAE-Sepharose CL-6B. Four peaks, designated A–D, were obtained (fig. 4b). Peak A contained unbound material; peak

B, material eluted with 0.1 M NaCl; peak C, material eluted with 0.3 M NaCl; and peak D, material eluted with 0.5 M NaCl. Only material from peak C, and not that from the other peaks, increased leakage of GOT and LDH from hepatocytes to a significant extent at low dose (10 mg/L) and caused a marked reduction in synthesis of albumin (table II). Dye exclusion tests demonstrated that material from peak C caused dose-dependent cytotoxic changes in hepatocytes, together with the increased leakage of GOT (fig. 5).

Phase-contrast microscopy showed that hepatocytes incubated for 24 h with material from peak C, in the concentration range of 1–5 mg/L, showed only a "rounding-up" (fig. 6a). However, higher doses of material from peak C (>20 mg/L) induced significant cell lysis within 24 h (fig. 6b), reducing the number of viable cells by $>90\%$.

Neutralisation tests

Each antiserum was titrated against glutaraldehyde-treated sheep red-blood cells coated with the corresponding antigen before use by the method described previously.²² Haemagglutination titres of all antisera exceeded 128. Pre-treatment of peak C with serial dilutions of antiserum to the LPS of

Fig. 3. Toxicity of graded doses of P65 material from *C. jejuni* GIFU 8734 (○—○) or *E. coli* 271-6 (●—●) for mouse hepatocytes. Cytotoxicity was determined in triplicate cultures after 24 h. The data were obtained from two separate experiments, and are expressed as the means for the six cultures.

Table I. Effect of P65 preparations on enzyme leakage and albumin synthesis in cultures of mouse hepatocytes

Sample*	Concentration (mg/L)	Released enzymes—mean (SD) mU/ml		Albumin synthesis mean (SD) mg/L
		GOT	LDH	
Medium control	...	6 (2)	18 (6)	2.2 (0.2)
P65 of <i>C. jejuni</i>	10	14 (3) ($p < 0.1$)†	26 (7) ($p < 0.1$)	2.1 (0.1)
	50	24 (8) ($p < 0.05$)	57 (0) ($p < 0.05$)	2.0 (0.1)
	100	59 (7) ($p < 0.01$)	116 (10) ($p < 0.01$)	1.6 (0.2) ($p < 0.1$)
P65 of <i>E. coli</i>	50	6 (3)	19 (5)	2.2 (0.2)
	100	8 (2)	20 (4)	2.1 (0.1)

*The hepatocytes were cultured with the concentrations of samples indicated for 24 h. Each culture was performed in triplicate and the data were obtained from three different experiments (i.e., nine cultures in total).

†The levels of significance for the observed values compared to the controls.

C. jejuni or the LT of *E. coli* did not abrogate the toxic effects, whereas antiserum to peak C neutralised its hepatotoxicity (fig. 7).

In-vivo effect of peak C

Intravenous injection of material from peak C at a dose of 10 µg/mouse induced marked elevation of the serum concentrations of GOT and glutamic-pyruvic transaminase (EC 2.6.1.2.:GPT) within 2 days (fig. 8). Serum concentrations of GOT and GPT were elevated more rapidly than those of LDH and alkaline phosphatase (EC 3.1.3.1.:ALP), and reached their maxima on the eighth day after injection. In contrast, LDH and ALP increased gradually and reached their maxima between the eighth and twelfth days. Serum concentrations of all four enzymes then fell gradually, but remained significantly elevated even 30 days after injection.

Most of the concentrations remained modestly elevated 3 months after injection.

Histological changes appeared rapidly after injection. The first pathological change was characterised by increased cytoplasmic staining intensity and pyknotic nuclei of hepatocytes (fig. 2b) and was observed in the parenchyma by 48 h after injection. Thereafter, the parenchymal changes gradually became localised and the foci of infiltrative lesions became necrotic (fig. 2c) by the twentieth day after injection. These pathological changes were similar to those observed after mice were infected intra-gastrically with living organisms.

Hepatotoxicity of clinical isolates

Among 20 clinical isolates, 14 persisted in the murine intestine 30 days after intra-gastric inocu-

Fig. 4. Chromatographic separation of hepatotoxic factor(s). (a) P65 material from *C. jejuni* GIFU 8734 chromatographed on Sepharose 4B: ●—●, optical density at 280 nm; •••, percentage cytotoxicity; V_o , void volume. (b) The second peak from Sepharose 4B chromatography was applied to a DEAE-Sepharose 4B column and eluted with an increasing NaCl gradient (---); †, initiation of gradient elution.

Table II. Effect of material from peak C on enzyme leakage and albumin synthesis by cultures of mouse hepatocytes

Samples	Enzyme activity released—mean (SD) mU/ml		Albumin synthesis mean (SD) mg/L
	GOT	LDH	
<i>C. jejuni</i>			
P65 (10 mg/L)	15 (2) (p < 0.1)	27 (5) (p < 0.1)	2.1 (0.2)
Peak A (10 mg/L)	7 (1)	15 (3)	2.2 (0.3)
Peak B (10 mg/L)	7 (2)	17 (2)	2.1 (0.1)
Peak C (10 mg/L)	70 (3) (p < 0.01)	164 (15) (p < 0.01)	1.2 (0.2) (p < 0.05)
Peak D (10 mg/L)	8 (3)	17 (3)	2.2 (0.2)
Medium control	7 (1)	16 (2)	2.3 (0.2)

See footnotes to table I.

lation (table III). Only eight of these also colonised the liver, and liver function tests disclosed that only four (Nara 24, KZ-6, MT-7 and DK-13) evoked hepatitis. P65 fractions prepared from the eight strains that colonised the liver were tested for hepatotoxicity *in vitro* and *in vivo*. The results are summarised in table IV. Only those fractions obtained from the four strains that caused hepatitis induced significant leakage of GOT and LDH 5 days after animals were given intravenous injections containing 100 μ g of the P65 preparations.

This capacity correlated well with cytotoxicity, as determined by the dye exclusion test.

Discussion

These results provide supportive evidence for our earlier view^{1,2} that *C. jejuni* can induce hepatitis in mice. The hepatotoxicity was attributable to sub-cellular components. These were enriched in the particulate fraction obtained by a 90-min centrifugation of lysed bacteria at 90 000g. The chromato-

Fig. 5. Toxicity of graded doses of peak C material for mouse hepatocytes. GOT leakage into the supernatant fluid and cytotoxicity for hepatocytes were assayed after 24 h in triplicate cultures. Data were obtained from three separate experiments, and are expressed as the mean (SD) for the 15 cultures; ●—●, percentage cytotoxicity; □, GOT (mU/ml).

Fig. 6. Effect of peak C material on the morphology of hepatocytes cultured for 24 h with: (a) peak C material, 5 mg/L; (b) peak C material, 20 mg/L; (c) LPS from *C. jejuni* GIFU 8734, 5 mg/L. Bar = 100 μ m.

Fig. 7. Protective effect of antiserum to peak C material. GOT leakage and cytotoxicity were assayed in triplicate cultures after 24 h and the results are expressed as the mean ($\pm$ SD) for the three cultures: cytotoxicity for mouse hepatocytes cultured with peak C material (10 mg/L) treated with an equal volume of serially diluted homologous antiserum (○—○), antiserum to *C. jejuni* LPS (○---○), or antiserum to LT from *E. coli* 271-6 (○-·-○); GOT leakage from hepatocytes treated with C material plus homologous antiserum (□), or with normal mouse serum (control) (■).

Fig. 8. Liver function tests in mice given 10 μ g of peak C material by intravenous injection. Three mice were examined per group at each time point and the results are expressed as the means of three samples. GOT, GPT and LDH are expressed in IU/L and ALP in KAU for mice given peak C material, 10 μ g (○), mice given LPS from *C. jejuni* GIFU 8734, 10 μ g (●), and mice given PB as controls (▲).

graphically-purified toxic factor (peak C) exhibited hepatotoxicity both *in vivo* and *in vitro*. A single intravenous injection of material from peak C at doses as low as 10 µg/mouse evoked hepatitis, as determined by liver function tests and histological studies. Material from peak C produced a focal infiltrative necrosis in the parenchyma, giving lesions that were very similar to those evoked in the livers of mice by intra-gastric administration of live bacteria.

Addition of material from peak C to hepatocyte cultures resulted in a significant, dose-dependent, increase in the leakage of GOT and LDH. Dye exclusion tests demonstrated that the severity of cytotoxicity correlated with the degree of enzyme leakage. Hepatocytes cultured with material from peak C at concentrations below 5 mg/L showed only a "rounding-up" during a 24-h culture. As the concentration of peak C material was increased, the rounded-up cells detached from plates and, finally, areas of cytolysis were observed in the monolayers. It is known that detachment of the cells in the culture dishes occurs as a very early event in hepatocyte damage during culture.²³ The

fact that peak C material induced an increase in leakage of GOT from hepatocytes at doses (1–5 mg/L) below those causing cell detachment suggests that the leakage may reflect changes in membrane permeability, not cell rupture. Higher concentrations of peak C material (>20 mg/L) induced marked leakage of GOT and also cytolysis. Here, enzyme leakage was due not only to permeabilisation of the cell membranes but also to rupture of the hepatocytes. Although it is known that enzyme leakage increases as a result of change in cell permeability^{24–26} the mechanisms of liver damage induced by the peak C material remains to be studied.

As previously reported,¹² some strains of *C. jejuni* can persist in the liver and gall bladder for a long period after intra-gastric inoculation. Organisms that have colonised the hepatobiliary tract can continue to release hepatotoxic factor(s). Since intra-gastric administration of peak C material did not induce hepatitis (data not shown), the factor may be released only from bacteria capable of colonising the hepatobiliary tract.

Twenty clinical isolates of *C. jejuni* were tested

Table III. Source details and pathogenicity of 20 clinical isolates of *C. jejuni*

Strain	Clinical manifestation		Pathogenicity in mice*		
	Stool	Fever	Intestinal† colonisation	Liver‡ colonisation	Hepatitis§
GIFU 8734	Reference strain		+	+	+
Nara-3	Watery	+	+	–	–
Nara-5	Watery	+	–	–	–
Nara-6	Watery	–	+	–	–
Nara-24	Bloody	+	+	+	+
Nara-42	Watery	–	–	–	–
Nara-68	Watery	+	–	–	–
Nara-69	Bloody	+	+	+	–
Nara-73	Bloody	–	+	–	–
Nara-77	Watery	+	+	–	–
Nara-79	Watery	–	+	–	–
KZ-4	Watery	–	–	–	–
KZ-6	Watery	–	+	+	+
KZ-9	Watery	+	+	+	–
KZ-12	Bloody	+	+	+	–
KZ-14	Watery	–	+	–	–
MT-6	Watery	+	–	–	–
MT-7	Asymptomatic		+	+	+
MT-9	Asymptomatic		+	+	–
MT-10	Watery	–	–	–	–
DK-13	Bloody	+	+	+	+

*Groups of five mice were infected intra-gastrically with 10⁶ cfu and bled 30 days after infection.

†+, More than four mice were found to carry 1000 cfu/g of intestinal tissue.

‡+, More than four mice were found to carry 1000 cfu/g of liver tissue.

§+, More than four mice exhibited serum GOT activities > 100 IU/L.

Table IV. Comparison of hepatotoxicity of P65 fractions prepared from eight strains of *C. jejuni* capable of colonising the mouse liver

Strain*	Serum enzymes— mean (SD) IU/L†		Percentage cytotoxicity‡— mean (SD)
	GOT	LDH	
GIFU 8734	158 (22)	394 (46)	58.4 (6.2)
Nara-24	142 (16)	400 (33)	56.5 (5.3)
Nara-69	60 (13)	128 (19)	9.8 (2.2)
KZ-6	162 (31)	402 (36)	60.3 (2.2)
KZ-9	62 (15)	131 (20)	10.2 (3.6)
KZ-12	58 (9)	126 (18)	9.8 (3.4)
MT-7	155 (18)	379 (26)	57.8 (4.2)
MT-9	56 (11)	125 (42)	8.6 (2.5)
DK-13	212 (32)	416 (42)	67.7 (5.9)

*Groups of five mice were given 100 µg of the P65 fractions from each strain by intravenous injection.

†Mice were bled 5 days after injection and the serum concentrations of GOT and LDH were measured; the experiment was done twice. Control GOT and LDH activities were 52 (11) IU/L and 110 (14) IU/L, respectively.

‡Cytotoxicity was assayed by the exclusion of trypan blue in a 24 h-culture of mouse hepatocytes dosed with P65 material 75 mg/L. Seven cultures were treated with each strains. Control samples (HBSS) caused 5.6 (2.2)% cytotoxicity.

for hepatotoxicity. Those strains that could colonise the liver did not always induce hepatocellular damage. However, strains capable of inducing liver damage could always colonise the liver. Hepatotoxic strains were isolated from asymptomatic patients.

It has not yet been ascertained whether the hepatotoxic factor is involved in the pathogenesis of campylobacter enteritis. However, it was found that material from peak C did not induce morphological changes in CHO and Y1 cells in preliminary experiments. Additionally, neither antiserum to the LPS of *C. jejuni* nor antiserum to the LT of *E. coli* neutralised the hepatotoxicity of peak C. These results indicate that the hepatotoxic factor(s) in peak C are different from the LPS of *C. jejuni* and from LT. Moreover, only antiserum to peak C material could prevent both the enzyme leakage from cultured hepatocytes and the morphological changes induced in hepatocytes by peak C.

C. jejuni has been recognised only as an enteropathogenic organism; however, several investigators have reported hepatitis following campylobacter enteritis.^{10,11} Nonetheless, only 20% of the strains examined here exhibited hepatotoxicity in mice. Preliminary experiments demonstrated that the hepatotoxic activity of peak C material was impaired by heating at 60°C for 30 min and by digestion with trypsin. Studies are now in progress to further purify peak C material to allow characterisation of the active component and its physico-chemical properties, and also to ascertain whether this factor can be involved in hepatitis associated with *C. jejuni* infections in man.

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