Cross-protection of infant mice against intestinal colonisation by *Campylobacter jejuni*: importance of heat-labile serotyping (Lior) antigens

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Summary. An association of the heat-labile antigens detected by the Lior serotyping scheme with ability to protect infant mice against gastrointestinal colonisation with *Campylobacter jejuni* has been established. Overall, 39 (57%) of 68 infant mice challenged with a heterologous strain of the same Lior serotype as the vaccine strain were protected, compared with 40 (85%) of 47 infants protected against a homologous challenge. In contrast, none of the infant mice challenged with a strain carrying the same heat-stable antigens (i.e., of the same Penner serotype as the vaccine strain) were protected.

Introduction

The infant mouse model (Field *et al.*, 1981) was used to investigate protection against colonisation of the intestinal tract by *Campylobacter jejuni*. Vaccination of a dam was shown by Dolby and Newell (1986) to prevent colonisation in offspring challenged with the same (homologous) strain, and to depend on the mammary secretion of the vaccinated dam, which contains high concentrations of IgG antibodies (Abimiku and Dolby, 1987). Although flagella are important in establishing infection in the intestinal tract of the infant mouse (Newell *et al.*, 1985), protection achieved by vaccination was shown to be independent of flagellar antigen (Dolby and Newell, 1986).

Our previous studies with animal models (Dolby and Newell, 1986; Abimiku and Dolby, 1987) demonstrated protection only against infection with the homologous strain of *C. jejuni*. In this study we investigated the relationships of heat-labile (LIO) antigens (Lior *et al.*, 1982) and heat-stable (PEN) antigens (Penner and Hennessy, 1980) in cross-protection experiments.

Materials and methods

Campylobacter strains

C. jejuni strain 81116 (NCTC 11828) PEN 6 LIO 6 and strain 53729 (NCTC 11626) PEN 27 LIO 23, obtained from the National Collection of Type Cultures, Colindale

Avenue, London NW9 5HT, were described previously (Dolby and Newell, 1986). Other strains used were 20186 PEN 6 LIO 11, 13024 PEN 29 LIO 6 and 18203 PEN 27 LIO 11 from the Public Health Laboratory, Withington Hospital, Manchester and strains CCUG 12066 LIO 6 and CCUG 15023 LIO 23 from the Culture Collection, University of Goteborg, Sweden, of unknown PEN serotype.

Protection and challenge experiments

Heat-killed (62°C) vaccine of *C. jejuni* 53729 was prepared as described previously (Dolby and Newell, 1986) from organisms grown under reduced oxygen tension at 37°C for 24 h on blood agar, harvested into phosphate-buffered saline (PBS), pH 7.2, and adjusted to 10 International Opacity Units (IOU) before heating at 62°C for 45 min. Live vaccine was prepared fresh for each use by resuspending *C. jejuni* strain 81116 grown on blood agar for 24 h in PBS, and adjusting to 5 IOU. Adult female mice received 0.2 ml intraperitoneally four times at weekly intervals.

On completion of vaccination mice were mated. Their infants, born 3–5 weeks later, were challenged orally 4 days after birth with 0.02 ml of suspensions of organisms grown on blood agar at 37°C for 24 h under reduced oxygen tension, harvested into Brucella Broth (Difco), adjusted to 10 IOU and diluted 1 in 2 in sterile skimmed milk. An inoculum of 0.02 ml contained 10⁷ viable organisms. The challenge strains were either the vaccine strain (homologous) or a heterologous strain matched with the vaccine strain for either PEN or LIO serotype antigens.

The degree of colonisation was estimated between 2 and 6 days after challenge, as described previously by Abimiku and Dolby (1987), by culturing segments of the

lower intestinal tract on selective and non-selective blood-agar media. A reduction in colonisation of 100-fold or more compared with the average for infants of non-vaccinated control dams was taken as evidence of protection. Samples of antiserum from dams taken 3-4 weeks after delivery and of milk from stomachs of 6-8 days old infant mice were collected as described by Abimiku and Dolby (1987). The serum samples were heated at 56°C for 30 min. and stored at 4°C. The milk was homogenised in PBS, centrifuged at 12 000 g and stored at -70°C for agglutination tests.

Agglutination tests

The slide agglutination technique for LIO antigen was adapted from that described by Lior *et al.*, (1982). Cultures were first examined for stability in suspension by emulsifying some of the growth in a drop of PBSA, pH 7.4, containing DNAase 0.02% w/v, then adding a drop of 2% NaCl and observing for autoagglutination. Rough cultures were subcultured and rechecked. For the test proper, a 10- μ l loopful of bacteria from a 24-h culture on a blood-agar plate was emulsified in DNAase-PBSA to give a suspension of 30 IOU, 20 μ l of which was then added to 20- μ l volumes of low dilutions (range 2 to 10) of antiserum and of milk in PBS in a microtitration tray. The tray contents were mixed thoroughly on a rotary shaker at room temperature and agglutination examined by eye after 2 min. PEN antigens were determined by the passive haemagglutination technique of Penner and Hennessy (1980) with sheep red blood cells sensitised with extracts of boiled *C. jejuni* against dilutions of antisera.

Results

Protection in infants challenged with the vaccine strains

The results of vaccination of dams with either live or heat-killed (62°C) vaccine before mating in preventing colonisation among their infants challenged with the vaccine strain are shown in table I. There was 76% protection with strain 81116 and 92% with strain 53729.

Table I. Protection of infant mice challenged with the vaccine strain

Vaccine strain	Number of litters	Number of infants	
		challenged	protected (%)
81116 (live)	6	21	16 (76)
53729 (heated)	8	26	24 (92)

Protection in infants challenged with strains of matched PEN serotype

All 26 infants of seven vaccinated dams examined after challenge with the heterologous strains 20186 (PEN 6 LIO 11) or 18203 (PEN 27 LIO 11) of the same PEN serotypes as the vaccine strains 81116 (PEN 6 LIO 6) or 53729 (PEN 27 LIO 23) respectively were colonised with *C. jejuni* to the level of 10⁶ cfu/mg of gut. Thus, none of the infants challenged with PEN matched strains were protected (table II).

Agglutinins to the vaccine strain measured by passive haemagglutination (PEN antigen) were detected in the sera of vaccinated dams at a titre of 20.

Protection in infants challenged with a strain of matched LIO serotype

Fifteen (43%) of the 35 infants challenged with strain 13024 (PEN 29 LIO 6) and 7 (64%) of the 11 infants challenged with strain CCUG 12066 (LIO 6), from 12 litters all vaccinated with live strain 81116 (PEN 6 LIO 6), were protected from colonisation (table III). These results compared favourably with the 76% of infants protected after a homologous challenge ($p > 0.1$). Seventeen (77%) of the 22 infants from five dams vaccinated with heated (62°C) strain 53729 (PEN 27 LIO 23) and

Table II. Protection of infant mice challenged with strains of the same Penner antigen type as the vaccine strain

Vaccine strain	Challenge strain	Number of litters	Number of infants	
			challenged	protected
81116 (live; PEN 6 LIO 6)	20186 (PEN 6 LIO 11)	4	15	0
53729 (heated; PEN 27 LIO 23)	18203 (PEN 27 LIO 11)	3	11	0

Table III. Protection of infant mice challenged with strains of the same Lior antigen type as the vaccine strain

Vaccine strain	Challenge strain	Number of litters	Number of infants	
			challenged	protected (%)
81116	13024 (PEN 29 LIO 6)	9	35	15 (43)
(live; PEN 6 LIO 6)	12066 (LIO 6)	3	64	7 (64)
53729	15023 (LIO 23)	5	77	17 (77)
(heated; PEN 27 LIO 23)				

challenged with strain CCUG 15023 (LIO 23) were protected, compared with the homologous protection of 92%.

Agglutinins to the LIO type antigens were present in the serum and milk of vaccinated mice at titres of 2–10 and 2 respectively with indicator suspensions of the homologous strain or a heterologous strain of matched LIO serotype.

Discussion

The data presented demonstrate that vaccinating dams with *C. jejuni* of the same LIO serotype as the challenge strain protects their young. Strains of *C. jejuni* vary in their ability to colonise or make good vaccines; although strain 53729 was heated (62°C) its ability to protect challenged infants equalled that of live 81116, and was by far superior to heated 81116 vaccine used at 10 IOU (Abimiku, unpublished data). LIO agglutinins were present in the sera and milk of vaccinated dams, and the presence

of LIO agglutinins and protection correlated well (table IV). Conversely, the PEN serotyping antigen did not seem to be involved in cross-protection, even though antibody could be detected in the sera of vaccinated dams by use of red blood cells sensitised with an extract of the vaccine strain. However, antibody titres detected were very low and we could not demonstrate antibodies to the PEN antigens of the heterologous challenge strain with the same PEN serotype as the vaccine strain. We cannot explain this observation.

The composition of the specific heat-labile antigens in the Lior typing scheme has not been fully characterised, though a flagellar protein of mol. wt. 62×10^3 has been found to be an essential determinant (Wenman *et al.*, 1985). The flagellum has been reported to be highly immunogenic (Kaldor *et al.*, 1983; Wenman *et al.*, 1985), but there is uncertainty as to whether sero-specificity is due to an antigen on the surface of the bacterial cell or a component of the flagellum. Some workers

Table IV. Correlation of Lior antigens with protection; summated results

Mouse group	Number of litters	Number of infants		LIO* agglutinin
		challenged	protected (%)	
(a) Homologous vaccine and challenge	14	47	40 (85)	+
(b) Heterologous vaccine and challenge with same PEN serotype†	7	26	0	—
(c) Heterologous vaccine and challenge with same LIO serotype†	17	68	39 (57)	+
(d) Non-vaccinated	11	35	0	—

*Agglutination of challenge strain by sera (diluted 1 in 5) of vaccinated dams.

†*C. jejuni* strains used for heterologous challenge as in tables II and III.

believe that serotype specificity disappears with the loss of flagellum (Wenman *et al.*, 1985). We have found that a non-flagellate strain did agglutinate in the Lior test but required 5 rather than 2 min. to do so. We were able to prevent colonisation in infant mice by vaccinating dams with the heat-killed (62°C) non-flagellate strain (Dolby and Newell, 1986; Abimiku and Dolby, 1987). This observation and the present findings suggest that Lior specificity and protection are not due entirely to the flagella. Newell (1985) suggested that a component of flagellar protein is exposed on the cell surface of non-flagellate strains.

It seems likely that whereas homologous protection in this model may involve antibody response to antigens found on both the flagella and the body of the organism, cross-protection may depend mainly on flagellar antigens, some of which cross-react with antigen(s) found on the surface of non-flagellate strains. Whether this cross-reacting anti-

gen can cross-protect by itself must await future experiments. Logan and Trust (1986) have already reported a cyanide bromide hydrolysis product of flagellin of mol. wt 4×10^3 contributing to sero-specificity. They also found that the flagellin carried many epitopes responsible for cross-reactions. Dunn *et al.* (1987) recently found flagellar material of mol. wt 63×10^3 showing amino acid substitutions and variations of charge. Thus, although we have demonstrated for the first time that cross-protection among strains of *C. jejuni* is possible with pairs of the same LIO serotype, it is by no means clear that this is exclusively due to flagellar protein. Much remains to be elucidated.

We thank Dr D. M. Jones and Mrs E. Sutcliffe of the Public Health Laboratory, Withington Hospital Manchester and Professor B. Kaijser of the University of Goteborg for providing typed strains of *C. jejuni*, the staff of the Animal House, the Division of Comparative Medicine of this Institute for help with the animals and Dr S. P. Borriello for helpful discussion.

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