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 LIO6 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, 
PpH 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-μl 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 μl of which was then 
added to 20-μl 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 
antiseras.

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

<table>
<thead>
<tr>
<th>Vaccine strain</th>
<th>Number of litters</th>
<th>Number of infants challenged</th>
<th>Number of infants protected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>81116 (live)</td>
<td>6</td>
<td>21</td>
<td>16 (76)</td>
</tr>
<tr>
<td>53729 (heated)</td>
<td>8</td>
<td>26</td>
<td>24 (92)</td>
</tr>
</tbody>
</table>

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 pro-
tected (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 
insects challenged with strain CCUG 12066 (LIO 
6), from 12 litters all vaccinated with live strain 
81116 (PEN 6 LIO 6), were protected from colonization (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

<table>
<thead>
<tr>
<th>Vaccine strain</th>
<th>Challenge strain</th>
<th>Number of litters</th>
<th>Number of infants challenged</th>
<th>Number of infants protected</th>
</tr>
</thead>
<tbody>
<tr>
<td>81116 (live; PEN 6 LIO 6)</td>
<td>20186 (PEN 6 LIO 11)</td>
<td>4</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>53729 (heated; PEN 27 LIO 23)</td>
<td>18203 (PEN 27 LIO 11)</td>
<td>3</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>
C. JEJUNI AGGLUTINOGENS AND MOUSE PROTECTION

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 |
|----------------|-----------------|------------------|------------------|----------------|
| 81116 (live; PEN 6 LIO 6) | 13024 (PEN 29 LIO 6) | 9 | 35 | 15 (43) |
| 12066 (LIO 6) | 3 | 64 | 7 (64) |
| 53729 (heated; PEN 27 LIO 23) | 15023 (LIO 23) | 5 | 77 | 17 (77) |

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). L10 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. 6 x 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

<table>
<thead>
<tr>
<th>Mouse group</th>
<th>Number of litters</th>
<th>Number of infants</th>
<th>Number of infants</th>
<th>LIO* agglutinin</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Homologous vaccine and challenge</td>
<td>14</td>
<td>47</td>
<td>40 (85)</td>
<td>+</td>
</tr>
<tr>
<td>(b) Heterologous vaccine and challenge with same PEN serotype†</td>
<td>7</td>
<td>26</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>(c) Heterologous vaccine and challenge with same LIO serotype†</td>
<td>17</td>
<td>68</td>
<td>39 (57)</td>
<td>+</td>
</tr>
<tr>
<td>(d) Non-vaccinated</td>
<td>11</td>
<td>35</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

*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 antigen 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 $4 \times 10^3$ 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 $63 \times 10^3$ 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. Kajser 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.

REFERENCES


