Mucosal and Systemic Immunity to Campylobacter jejuni in Rabbits after Gastric Inoculation

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Received 24 June 1987/Accepted 30 September 1987

The mucosal and systemic immune responses to Campylobacter jejuni were studied in rabbits receiving gastric inoculation with live organisms. A lavage procedure was used to facilitate repeated monitoring of the intestinal immune response to C. jejuni. Immunity to C. jejuni was determined by secondary challenge by using the removable intestinal tie adult rabbit diarrhea (RITARD) model and monitoring for resistance to colonization and bacteremia. Oral-gastric inoculation of normal rabbits produced a transient intestinal colonization without diarrhea. C. jejuni serotypes differed in their ability to colonize the intestines of rabbits and to stimulate primary intestinal and serum antibody responses. Animals previously colonized were resistant to recolonization and the development of bacteremia after homologous challenge by the RITARD procedure but were not resistant to heterologous challenges. Anticampylobacter intestinal and serum IgA titers before this secondary infection were the most likely predictors of resistance to colonization and bacteremia.

Campylobacter jejuni is a major cause of infectious diarrhea, but little is known regarding its virulence factors or immunogenicity (29). Prospective epidemiological and human challenge studies after prior infection suggest that protective immunity develops after C. jejuni infection and that vaccination for campylobacter enteritis is possible (1, 12). Human volunteers fed C. jejuni become infected and respond with the development of specific serum immunoglobulin G (IgG), IgM, and IgA to campylobacter (1). When 20 adult volunteers were fed 108 or 109 organisms, diarrhea developed in 9 of these 20. When 7 of these volunteers who had been ill were rechallenged later with the same organism, none developed disease, whereas 6 of 12 nonimmunized volunteers developed diarrhea.

Immunity has also been demonstrated in an animal model. When normal rabbits were infected by gastric inoculation with C. jejuni, some had bacteremia, whereas all became transiently colonized without developing diarrhea (7). After oral inoculation, these rabbits became resistant to intestinal colonization and bacteremia when challenged with the same strain by the removable intestinal tie adult rabbit diarrhea (RITARD) procedure, which causes a mucus-containing diarrhea, bacteremia, and an abnormal intestinal histopathology which mimics the human disease in normal rabbits (8). Rabbits challenged initially with this procedure and then rechallenged to 5 to 8 weeks later develop an intestinal IgA and serum IgG and IgM immune response to C. jejuni and a greater resistance to recolonization (24).

The development of anticampylobacter antibodies with age in endemic areas suggests that protective immunity in humans reflects the appearance of a specific anticampylobacter serum or intestinal antibody response. Titers of campylobacter-specific serum IgA, IgG, and IgM have been shown to progressively increase with age in children from Thailand and Bangladesh (2, 5). The development of campylobacter immunity in young children living in these endemic areas may explain the frequent recovery of C. jejuni from apparently healthy older children and also the age-associated decrease in the C. jejuni illness/infection ratio found in Bangladesh and in other developing countries (12). These researchers concluded that the age-related increase in anticampylobacter serum IgA documented in Bangladeshi children correlated with the acquisition of protective immunity and that the development of a specific mucosal IgA response was responsible for protection.

The purpose of the present study was to use the oral immunization-RITARD challenge system in the rabbit as a model to characterize the campylobacter-specific immune responses associated with resistance to colonization and protection against bacteremia when animals are infected with this organism. The time course of the systemic and mucosal IgG and IgA immune response of rabbits after primary and secondary infection with C. jejuni was studied to determine its specificity and predictive value as an index of immunity against campylobacter infection. The mucosal immune response was monitored with intestinal secretions obtained by a lavage procedure adapted to the rabbit, which facilitated the repeated sampling of intestinal secretions for campylobacter-specific antibody. Such studies are needed to understand the immunological resistance mechanisms which will be required for the evaluation of future experimental vaccines before their testing in humans.

MATERIALS AND METHODS

Bacterial cultures. C. jejuni HC (Penner serotype 27) was isolated at the Naval Hospital, Bethesda, Md., from the blood of a patient with enteritis. Strain 39-3 (Penner serotype 2:42) was an isolate from a calf stool. It was used to infect a rabbit by the RITARD procedure, and the isolate used in this study was recovered from the spleen. Strain E8 (Penner serotype 34;26w) was isolated in Alexandria, Egypt from the

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stool of an infant with acute diarrhea. Frozen stocks were thawed, inoculated on tryptic soy blood agar plates (Remel, Lenexa, Kan.), and incubated at 42°C in polybags (Levi-Bros. Paper Co., Chicago, Ill.) with an atmosphere of 85% N₂ - 10% CO₂ - 5% O₂. After 18 h of incubation, cells were suspended in brucella broth supplemented with 0.04% cysteine and 0.25% serine to a concentration giving an optical density at 625 nm of 0.05. The resulting suspension (6 ml) was overlaid in 25-cm² T flasks (Becton Dickinson Labware, Oxnard, Calif.) containing 4 ml of brucella blood agar. After 18 h of incubation at 37°C, the broth phase portion of the biphasic culture system was collected, screened for contamination by phase-contrast microscopy, pooled, and used directly for animal challenge.

Animal challenge. Female New Zealand White rabbits (Dutchland Laboratories, Denver, Pa.), weighing 1.5 to 2.0 kg, were fed 15 ml of the bacterial suspension after neutralization of gastric acidity (10). Rabbits were challenged by the RITARD procedure as previously described (8, 26). All challenge doses, monitored by plate counts, were approximately 10¹⁰ organisms per animal.

Monitoring of infection. After oral and RITARD challenge, rectal swabs from the rabbits were cultured for C. jejuni by direct plating on C. jejuni selective agar (Remel). To enumerate C. jejuni in fecal samples or lavage secretions, specimens were homogenized and serial 10-fold dilutions were made in brucella broth. Dilutions were spread on selective media. Viable counts were determined by plate count, and CFU per gram (wet weight) of feces were calculated. Representative colonies were confirmed as C. jejuni by morphology, catalase and oxidase reactions, naldixic acid sensitivity, cephalothin resistance, and growth at 42 but not at 25°C. Blood specimens (2 ml), obtained by cardiac puncture, were cultured for viable bacteria in vented tryptic soy broth blood culture bottles (GIBCO Laboratories, Lawrence, Mass.). Blood culture bottles were incubated at 37°C and subcultured on brucella blood agar plates at 2, 5, and 7 days postinoculation to screen for bacterial growth.

Lavage procedure. Intestinal secretions were collected by a lavage procedure recently adapted to the rabbit (6). Briefly, rabbits were anesthetized with 70 mg of ketamine per kg and 0.4 mg of acepromazine per kg, and at 10-min intervals, rabbits were washed with 15 ml of an isotonic salt solution. At 30 min after the last feeding, rabbits were injected intraperitoneally with 4 mg of pilocarpine per kg and placed back in their cages while still sedated. The steady intestinal discharge which occurred over the next 10 to 40 min was collected in petri dishes placed under the rabbit. Intestinal secretions from individual animals were then expressed through gauze to remove large fecal material and processed immediately or held at -20°C. During lavage processing, potential protease activity was inhibited by heat treatment at 56°C for 15 min. Secretions were then diluted 1:5 in phosphate-buffered saline-Tween, supplemented with soybean trypsin inhibitor and Merthiolate to further suppress protease activity and inhibit bacterial growth, clarified by centrifugation, filtered through a 0.45-µm-pore-size filter, and stored at -20°C until assayed. Serum samples were obtained while rabbits were still sedated.

Antigen preparation for enzyme-linked immunosorbent assay (ELISA). C. jejuni flagellar antigen was prepared as previously described (16), except that the acid dissociation step was omitted. C. jejuni cells were grown on Mueller-Hinton agar at 37°C for 48 h. Cells were harvested in distilled water and homogenized in a blender with three 30-s bursts. All further processing was at 4°C. Homogenates were centrifuged at 10,000 × g for 1 h to remove cells and large debris. The supernatant was collected, and the pellet was suspended in distilled water and recentrifuged at 10,000 × g for 1 h. The two supernatants were pooled and centrifuged at 100,000 × g for 1 h. The resulting pellet was suspended in 10 mM Tris (pH 7.2) and centrifuged at 10,000 × g for 1 h to remove any remaining bacterial material. The supernatant was collected and centrifuged at 100,000 × g for 1 h. The pellet, representing crude flagellar protein, was suspended in a small volume of Tris buffer and stored at -80°C until used. The protein concentration of flagellar preparations was determined by the Bio-Rad assay (Bio-Rad Laboratories, Richmond, Calif.) with bovine serum albumin as the reference standard. This procedure produced preparations enriched for flagellar filaments, along with several major outer membrane proteins. In sodium dodecyl sulfate-polyacrylamide electrophoresis gels, the major proteins present migrated with apparent molecular weights of 29,000, 31,000, 45,000, and 62,000.

Isotype-specific goat anti-rabbit immunoglobulin reagents. Goat anti-rabbit IgA and IgG were obtained by immunizing goats with purified rabbit colostral IgA (9) or rabbit serum IgG, respectively. Each antiserum was rendered specific by absorption with immunoglobulin fractions coupled to Sepharose 4B with cyanogen bromide and then immunospecifically purified with a homologous affinity column. The isotype specificity of goat anti-rabbit IgA and IgG were determined by indirect ELISA. Microtiter ELISA plates were coated with 0.2 µg of rabbit IgA or IgG per well. Dilutions of goat anti-rabbit IgA or IgG were added, and binding to the plate was detected with alkaline phosphatase-conjugated swine anti-goat IgG (TAGO, Burlingame, Calif.). The substrate reaction was carried out with p-nitrophenylphosphate for 10 min and measured at 405 nm. The immunospecific antiserum were then labeled with horseradish peroxidase (Kirkegaard and Perry Laboratories, Gaithersburg, Md.). For measuring C. jejuni-specific antibodies, horseradish peroxidase-conjugated goat anti-rabbit IgA and IgG were used at appropriate dilutions to remove any cross-reactivity.

Anticampylobacter antibody ELISA procedure. Campylobacter-specific IgA and IgG antibodies were measured in rabbit sera and intestinal secretions by ELISA (6). Microtiter ELISA plates were coated with 0.2 µg of rabbit IgA or IgG per well. Dilutions of goat anti-rabbit IgA or IgG were added, and binding to the plate was detected with horseradish peroxidase-conjugated goat anti-rabbit IgA or IgG. The substrate reaction was carried out with ABTS (2,2′-azino-di-[3-ethyl-benzthiazoline sulfonate]) for 1 h and measured at 414 nm. Datum points represent geometric mean titer. Endpoint titers were expressed as the reciprocal dilution giving absorbance values greater than three standard deviations above background absorbance in wells containing no antigen.

Study protocol. One week before rabbits were fed, lavage and serum samples were obtained from all animals and assayed to determine baseline serum and intestinal anticampylobacter antibody titers. One week later (week 0), animals were immunized by oral feeding. Lavage and serum samples were obtained at weekly intervals postfeeding (weeks 1, 2, and 3). At 4 weeks, animals were challenged by the RITARD procedure. Serum and lavage samples were collected again during the 2 months after the RITARD challenge (weeks 5 through 12). Homologous challenge studies involved oral feeding, followed by challenge by the RITARD procedure with the same Penner serotype, whereas
heterologous challenge studies involved oral feeding and challenge by the RITARD procedure with different Penner serotypes. Control animals were fed sterile broth at the time of oral challenge.

RESULTS

Anticampylobacter antibody response after primary and secondary challenge with C. jejuni. The time course of anticampylobacter intestinal and serum antibody responses after primary oral immunization and secondary challenge with either homologous or heterologous serotypes was studied. Groups of rabbits were inoculated intragastrically with C. jejuni strains and then challenged approximately 1 month later by the RITARD procedure. One week before gastric feeding and subsequently at weekly intervals after primary and secondary infection, intestinal lavage secretions and serum samples were collected and assayed for campylobacter-specific IgA and IgG antibodies. Primary and secondary intestinal (IgA) and serum (IgA and IgG) antibody response kinetics were determined for all three study serotypes.

However, only the time courses for primary mucosal and serum responses to the HC and E8 strains and secondary responses to HC RITARD challenge are shown in Fig. 1. These specific response patterns were selected because they were representative of those observed with other immunization-challenge combinations involving the C. jejuni serotypes under study here.

C. jejuni strains differed in their ability to stimulate a primary mucosal and serum antibody response (Fig. 1). Anticampylobacter IgA (lavage and serum) and IgG (serum) titers in HC-inoculated rabbits were significantly higher than those detected in their E8-inoculated counterparts (compare A to C versus D to F for weeks 1 through 3 postinoculation [Fig. 1]). In HC-inoculated rabbits, the intestinal IgA response developed before the serum antibody response and was of considerably shorter duration (Fig. 1, A to C). The kinetics of the secondary intestinal IgA response in HC-inoculated rabbits challenged with HC by the RITARD procedure were similar to the primary response in these animals (Fig. 1A). The kinetics of the secondary intestinal and serum antibody responses to the crude C. jejuni flagellar antigens in HC-inoculated rabbits rechallenged with either

![Graphs illustrating antibody response kinetics](http://iai.asm.org/)
the E8 or 39-3 strains were similar to those seen in the HC homologous challenge group (data not shown). Intestinal and serum antibody response patterns in animals inoculated with strain 39-3 and then challenged with the same or a heterologous strain were again similar to those observed after HC inoculation (data not shown). In contrast to the HC- and 39-3-inoculated groups, animals inoculated with E8 mounted only a weak antibody response after primary challenge (Fig. 1, D to F). Suprisingly, these animals were primed for a stronger secondary response (compared with their HC- or 39-3-inoculated counterparts) when challenged with the HC strain (compare A to C versus D to F for weeks 5 through 12 [Fig. 1]). The immune responses in rabbits inoculated with E8 and challenged with E8 or 39-3 were similar to those observed in the HC-challenged animals (data not shown). The antibody responses in the two challenge groups (Fig. 1) also differed in the degree of cross-reactivity to the two crude flagellar antigens used in the present study. The secondary antibody response after heterologous challenge with the RITARD procedure showed much greater cross-reactivity for the flagellar antigens of both challenge serotypes compared with the antibody response after homologous challenge (compare A to C versus D to F [Fig. 1]). In both the homologous and heterologous challenge groups, no anticampylobacter IgG was detected in intestinal secretions after either primary or secondary infection.

C. jejuni intestinal colonization after primary and secondary infection with homologous and heterologous serotypes. To determine whether colonization with C. jejuni protected animals against recolonization, separate groups of rabbits were first inoculated via a nasogastric tube with one of the three strains of C. jejuni under investigation and monitored for intestinal colonization by rectal swab. As in earlier studies (7), rabbits given C. jejuni for the first time became colonized without signs of diarrhea. Based on the total number of rabbits intragastrically inoculated with a given strain in the present study, the mean duration of primary colonization for the HC (n = 25), E8 (n = 18), and 39-3 (n = 17) strains were 13.2 ± 4.4, 18.1 ± 5.4, and 21.4 ± 4.3 days, respectively. Only the HC strain differed significantly in its ability to colonize nonimmune rabbits, since rabbits infected intragastrically with HC were colonized for fewer days than were groups challenged with either E8 or 39-3. While colonized, all groups shed similar numbers of organisms in their fecal pellets. Campylobacter CFU per gram of feces peaked at 3 to 6 days postfeeding, when all groups shed approximately 10^6 to 10^7 organisms per g. Fecal shedding then diminished over the following weeks, with the HC challenge group exhibiting the most rapid clearance.

At 1 month after oral immunization, when all animals were no longer colonized with C. jejuni, rabbits were challenged with the RITARD procedure with either the same organism or a heterologous serotype. The mean number of days that rabbits were colonized after the oral immunization and the challenge with the RITARD procedure is shown in Fig. 2. In all three homologous challenge groups (Fig. 2A), the duration of colonization after challenge by the RITARD procedure was significantly shorter in previously immunized animals than in control animals given only sterile broth before RITARD challenge. In all groups, the majority of rabbits had negative fecal swabs by 2 days after reinoculation with the RITARD procedure. In the HC group, 4 of 12 rabbits had negative fecal cultures on day 1 postchallenge, with the remaining animals becoming negative by day 2. Although colonization resistance occurred when rabbits were challenged with the homologous strain, animals were not resistant to recolonization when challenged with either of the two heterologous serotypes (Fig. 2B). The duration of colonization in rabbits challenged with a heterologous serotype was the same as that determined for broth-inoculated animals subsequently challenged by the RITARD procedure with the same serotypes (Fig. 2A, broth-inoculated controls).

Incidence of bacteremia in C. jejuni-immunized animals. The incidence of bacteremia was compared in orally immunized and control rabbits challenged by the RITARD procedure (Table 1). In nonimmune animals, the HC strain exhibited the greatest potential for bacteremia. At 24 h postchallenge, 11 of 13 rabbits (85%) in the HC-infected group were bacteremic. Although the numbers per group are smaller, only 20 and 40% of the animals infected with E8 or 39-3, respectively, became bacteremic. Rabbits immunized with C. jejuni varied in their resistance to bacteremia when

![Graph A](https://via.placeholder.com/150)

![Graph B](https://via.placeholder.com/150)

**FIG. 2.** Mean number of days rabbits were colonized after intragastric inoculation and RITARD challenge with homologous (A) or heterologous (B) serotypes. The horizontal axis showing the various challenge groups identifies the initial feeding strain (□) and the RITARD strain (●). B challenge groups (panel A) consist of rabbits initially inoculated with sterile culture broth and subsequently challenged by the RITARD procedure with the indicated C. jejuni strains. The numbers of animals included in each challenge group are given above the bars. An asterisk indicates a significant difference from nonimmunized controls (B challenge group) inoculated with broth and challenged (Student's t test; *P* < 0.05).
TABLE 1. Incidence of bacteremia in normal and immunized rabbits 24 h after challenge with C. jejuni serotypes via the RITARD procedure

<table>
<thead>
<tr>
<th>Challenge group and oral immunization strain/RITARD challenge strain</th>
<th>No. bacteremic/no. tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonimmune* B/HC ..................................................</td>
<td>11/13 (85)</td>
</tr>
<tr>
<td>B/E8 ...........................................................................</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>B/39-3 .....................................................................</td>
<td>2/5 (40)</td>
</tr>
<tr>
<td>Homologous H/C/HC ................................................................</td>
<td>0/12 (0)</td>
</tr>
<tr>
<td>E8/E8 ...........................................................................</td>
<td>0/7 (0)</td>
</tr>
<tr>
<td>39-3/39-3 ...................................................................</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td>Heterologous E8/HC ................................................................</td>
<td>3/5 (60)</td>
</tr>
<tr>
<td>39-3/HC ........................................................................</td>
<td>5/6 (83)</td>
</tr>
<tr>
<td>E8 ..............................................................................</td>
<td>1/6 (17)</td>
</tr>
<tr>
<td>39-3/E8 .......................................................................</td>
<td>3/6 (50)</td>
</tr>
<tr>
<td>HC/39-3 ........................................................................</td>
<td>1/6 (17)</td>
</tr>
<tr>
<td>E8/39-3 .......................................................................</td>
<td>2/6 (33)</td>
</tr>
</tbody>
</table>

* Rabbits in this challenge group were initially inoculated with sterile culture broth before RITARD challenge with the indicated C. jejuni strains.

challenged with homologous or heterologous serotypes. Rabbits given gastric inoculations and challenged with the same serotype had no bacteremia, whereas the frequencies of bacteremia in animals challenged with a heterologous serotype were not significantly different than those observed in nonimmune animals.

Anticampylobacter antibodies as indicators of protective immunity. The association between pre-RITARD challenge antibody levels and subsequent resistance to intestinal colonization and bacteremia is shown in Table 2. Rabbits immunized by gastric inoculation with HC, E8, 39-3, or sterile broth and then challenged with strain HC via the RITARD procedure were used for this determination, since this group provided the largest number of animals for analysis. Specific risk ratios were calculated on the basis of intestinal or serum antibody titers to the HC strain of C. jejuni, determined 7 days before RITARD challenge with this organism. The risk of colonization or bacteremia with the HC strain decreased significantly as intestinal and systemic HC antibody titers increased. Pre-RITARD lavage IgA and serum IgA levels to the challenge organism were the strongest predictors of resistance. Rabbits with lavage and serum IgA titers in the 50 to 99 range were significantly more resistant to colonization with HC than were rabbits in the <50 titer group. Higher titers of lavage and serum IgA (≥100) were associated with protection of rabbits against both colonization and bacteremia. Substantially higher levels of serum IgG (titers of ≥1,600) were associated with similar levels of resistance.

DISCUSSION

Our results indicate that the oral immunization-RITARD challenge system is an effective model for the study of anticampylobacter immunity in rabbits. Animals exposed previously to C. jejuni were resistant to recolonization and bacteremia upon challenge with the homologous organism but not when they were challenged with a heterologous organism. For C. jejuni HC, the risk of colonization and bacteremia in rabbits challenged with HC was significantly greater in animals having low circulating titers of HC-specific antibody (Table 2). Anti-HC intestinal IgA titers before challenge were the strongest predictors of resistance to colonization. These results complement and expand on the previous observations of Ruiz-Palacios and co-workers (24), who first presented evidence suggesting that intestinal IgA antibody played an important role in anticampylobacter immunity in the rabbit model.

The results of these animal studies appear to parallel observations of human campylobacter infections. Seroepidemiological studies of campylobacteriosis in developing countries and immunological characterization of patients experiencing chronic or recurrent C. jejuni infections also have indicated that IgA plays an important role in immunity to this organism. In Bangladesh, the age-related increase in anticampylobacter serum IgA titers noted among children and young adults appeared to be the best indicator of campylobacter immunity (2, 12). In addition, individuals with documented IgA immunodeficiencies have frequently been reported to have problems recovering from C. jejuni enteritis.

TABLE 2. Pre-RITARD challenge antibody levels in rabbits versus their relative risk of colonization and bacteremia when challenged with C. jejuni HC by the RITARD procedure

<table>
<thead>
<tr>
<th>Antibody to HC</th>
<th>Pre-RITARD antibody titer*</th>
<th>No. of animals/titer group</th>
<th>Challenge results**</th>
<th>Relative risk*** of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. colonized (%)</td>
<td>No. bacteremic (%)</td>
</tr>
<tr>
<td>Lavage IgA</td>
<td>≤50</td>
<td>16</td>
<td>16 (100)</td>
<td>12 (75)</td>
</tr>
<tr>
<td></td>
<td>50–99</td>
<td>4</td>
<td>2 (50)</td>
<td>2 (50)</td>
</tr>
<tr>
<td></td>
<td>≥100</td>
<td>6</td>
<td>1 (17)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Serum IgA</td>
<td>≤50</td>
<td>19</td>
<td>19 (100)</td>
<td>15 (79)</td>
</tr>
<tr>
<td></td>
<td>50–99</td>
<td>2</td>
<td>1 (50)</td>
<td>1 (50)</td>
</tr>
<tr>
<td></td>
<td>≥100</td>
<td>6</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Serum IgG</td>
<td>&lt;800</td>
<td>15</td>
<td>15 (100)</td>
<td>12 (80)</td>
</tr>
<tr>
<td></td>
<td>800–1,599</td>
<td>4</td>
<td>3 (75)</td>
<td>2 (50)</td>
</tr>
<tr>
<td></td>
<td>≥1,600</td>
<td>8</td>
<td>2 (25)</td>
<td>2 (25)</td>
</tr>
</tbody>
</table>

* Prechallenge intestinal and serum antibody titers were determined by isotype-specific ELISA. The values shown are titers to the HC strain of C. jejuni determined 7 days before RITARD challenge.

** Colonization was determined by rectal swabs taken at 48 h post-RITARD, and bacteremia was determined by blood cultures taken at 24 h post-RITARD.

*** Relative risk = percent in titer group colonized or bacteremic/percent in lowest titer group colonized or bacteremic. A risk ratio (18) of 1.0 indicates a high relative risk of either colonization or bacteremia, whereas a risk ratio of 0 indicates minimal relative risk.

**** Boldface values indicate risk ratios significantly different (P < 0.05) from the lowest titer group by the Fischer exact test.
jejuni strains differed in their response in experimentally infected rabbits was similar to that observed in human patients recovering from campylobacter enteritis (3, 13). As in the rabbit, the human serum IgA and IgG responses develop rapidly after disease onset. The human campylobacter IgA antibody titer generally peaks at approximately 10 days postonset and then declines relatively quickly, whereas IgG titers may not peak until 3 to 4 weeks postinfection and subsequently persist for several weeks.

The results from the present study demonstrate that campylobacter-specific serum and intestinal antibodies develop after primary challenge by oral inoculation and that C. jejuni strains differed in their ability to stimulate primary mucosal and systemic antibody responses. These studies were facilitated by the lavage procedure, which permitted repeated sampling of intestinal secretions for campylobacter-specific IgA. Rabbits immunized perorally with strain HC had detectable intestinal IgA titers 1 week postinoculation, which then declined quickly over the next 2 weeks. In contrast, the intestinal IgA response to strain E8 was significantly lower (compare A to C versus D to F for weeks 1 to 3 (Fig. 1)). These strain-specific response differences were intestinal, since E8 actually colonized more effectively than did HC (Fig. 2). Prolonged colonization by E8 compared with HC may be due to the lower intestinal IgA response induced by E8 compared with HC.

Differences in the immune responses induced by campylobacter may relate to adherence or invasive characteristics of the organism. In vitro binding studies have shown that HC attaches more readily to INT 407 cells and rabbit intestinal mucus than does E8 (21). Within the gut, HC could associate more closely with the intestinal mucosa than does E8, thus more effectively stimulating the mucosal immune system. The higher incidence of bacteremia detected in nonimmunized rabbits infected with the HC strain via the RITARD procedure (Table 1) could also indicate that HC and E8 differ in their invasiveness. Since campylobacter enteritis may vary clinically from a watery diarrhea to a dysenterylike bloody diarrhea, it has been suggested that certain strains may have a greater potential for intestinal epithelial invasion than others (29). However, differences in susceptibility to the bactericidal activity of serum (4) could also contribute to differences in the incidence of bacteremia.

Both groups developed a detectable secondary intestinal IgA response after challenge. However, the secondary response in the E8/HC challenge group (which was inoculated with strain E8 and later challenged with strain HC) was of a greater magnitude and duration than that observed in the HC/HC group. The lack of a marked secondary response in the homologous challenge group is similar to that observed in immunized rabbits undergoing rechallenge with Shigella flexneri (14) or Vibrio cholerae (23). For V. cholerae, the investigators suggested that the antibacterial immunity induced by the initial infection interfered with mucosal colonization and efficient antigenic stimulation after secondary infection.

Our findings indicate that prevention of C. jejuni colonization in immune animals may be due to the action of IgA antibody. This molecule prevents V. cholerae (11) and enterotoxigenic Escherichia coli (19) adherence to intestinal epithelium, and anticampylobacter IgA in the gut may also afford protection by preventing the interaction of these organisms with intestinal epithelial cells. McSwegan et al. (20) have shown that C. jejuni is capable of binding to intestinal epithelial cells in vitro. When cultured epithelial cells in this assay system were overlaid with mucus gel preparations from nonimmune rabbits, campylobacter bind-

ing was only marginally reduced. However, when intestinal mucus from orally immunized rabbits was used, binding was significantly inhibited. ELISA analysis of immune mucus preparations indicated that the preparations were rich in anticampylobacter IgA and absorption with whole cells of C. jejuni removed these specific antibodies. Absorbed immune mucus preparations were no longer able to block campylobacter epithelial cell binding. In similar experiments, it was shown that mucus from nonimmune rabbits could be made to block campylobacter binding in the in vitro cell assay if it were first premixed with intestinal secretions (lavage fluids) from immune rabbits shown to contain anticampylobacter IgA. Antibody-containing mucus preparations could reduce binding to epithelial cells by several mechanisms. Antibodies could block adhesins on the bacterial surface, agglutinate the campylobacter cells, or interfere with chemotaxis toward receptor sites (19, 20).

The importance of the cell surface as a determinant of bacterial pathogenicity is well recognized (25). Surface components of C. jejuni have been extensively studied to determine their relative contributions to the pathogenicity and immunogenicity of this organism (16, 29). The 62,000-molecular-weight bacterial outer membrane protein (30) appears to be the major antigens recognized by serum antibodies from infected humans and animals. Western blots (immunoblots) of intestinal IgA antibodies, extracted from fecal samples of patients recovering from C. jejuni enteritis, also reacted with the flagellar antigen and the major outer membrane protein (30).

Further studies are needed to determine the specificity and functional properties of the protective IgA antibodies present in the gut of the immunized animals. The presence of common surface antigens among C. jejuni strains may explain the observed cross-reactivity in the antibody responses detected in the present study. The crude antigen preparations used in ELISA studies were enriched for flagellar and major outer membrane protein antigens. Epitopic analysis indicates that both possess group- or species-specific determinants, whereas the flagellar protein also possesses epitopes with strain or serotype specificity (17). From the heterologous challenge results, it appears that the common antigens shared by the different C. jejuni strains may not have been responsible for the protective immunity observed in the homologous challenge groups. Although intestinal IgA antibodies were directed against flagellar proteins, other non-flagellum-associated campylobacter antigens may also be important in mucosal gut immunity. For example, in the mucus inhibition assay (20), absorption of the immune lavage fluid with purified flagella, before mixture with mucous gel from nonimmune rabbits, had no significant effect on the ability of these preparations to block C. jejuni adherence to epithelial cells. The importance of other antigens in the protective immune response is also indicated by the observation that E8-inoculated rabbits are resistant to homologous challenge without responding with high titers to the antigen preparation used in the ELISA reactions presented here.

From the results of the present study, it would appear that the immune response to campylobacter infection in rabbits is analogous to the response observed in humans after infection with this organism. This study, as well as the work of several other investigators, suggests that immunity in humans and rabbits is associated with specific anticampylobacter intestinal IgA antibodies (2, 12, 24). In addition, the kinetics of the intestinal IgA response in campylobacter-infected rabbits were strikingly similar to those observed in
human after natural infection with *C. jejuni* (15) and other enteric pathogens, such as enterotoxigenic *E. coli* (27) and *V. cholerae* (28). Thus, the rabbit model appears to be an excellent one for evaluating the antigen-specific responses associated with protective mucosal immunity to *C. jejuni*. This information is essential to the development of the immunological criteria needed for the formulation and evaluation of future campylobacter vaccine candidates.

**ACKNOWLEDGMENTS**

We thank J. Macmillan, M. Flynn, and J. Eckstein for valuable advice and information; Carole Cole and Darren T. Kerner for excellent technical assistance; and the members of the Laboratory Animal Sciences Department for their invaluable assistance.

This study was supported by the Naval Medical Research and Development Command, Bethesda, Md., work unit no. 62770A3M 162770AB70A5309.

**LITERATURE CITED**


