Detection of Immunoglobulin A in Urine Specimens from Children with Campylobacter-Associated Diarrhea by a Chemiluminescent Indicator-Based Western Immunoblot Assay

SHUENN-JUE WU,1,* GARY PAZZAGLIA,2 RICHARD L. HABERBERGER,3 JOHN J. OPRANDY,1 DONNA G. SIECKMANN,1 DOUG M. WATTS,1 AND CURTIS HAYES1

Infectious Disease Threat Assessment1 and Enteric Diseases2 Programs, Naval Medical Research Institute, Bethesda, Maryland 20889-5035, and U.S. Naval Medical Research Unit No. 2, Jakarta 10110, Indonesia2

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A Western blot (immunoblot) assay was used to detect Campylobacter-specific immunoglobulin A in urine. Acute-phase urine samples from six children with Campylobacter diarrhea had titers ranging from 2 to 8. The highest titer was detected 4 days postonset. Campylobacter-specific immunoglobulin A was undetectable in the paired convalescent-phase specimens and urine samples from three control children.

Campylobacter spp. are an important cause of infectious diarrheas worldwide, affecting persons of all ages in both industrialized and developing nations (2). In tropical countries, Campylobacter-associated diarrhea is a major health problem among children (7). In clinical laboratories, a definitive diagnosis of Campylobacter sp. infection normally requires 3 days by bacteriological and biochemical procedures. A rapid enzyme-linked immunosorbent assay has been used to detect Campylobacter-specific immunoglobulin A (IgA) in sera, urine, and stool extracts (3). Western blotting (immunoblotting) techniques have also been used to demonstrate Campylobacter-specific antibodies in sera and stool specimens from patients (5, 8). The current study was undertaken to investigate the usefulness of Western blot analysis of urine specimens for early and rapid diagnosis of Campylobacter infection.

Study participants were Indonesian children who had been hospitalized because of acute diarrhea. Fecal specimens were obtained from the patients on day 1 of hospitalization and cultured for Campylobacter spp. by bacteriological techniques. Urine was obtained from each Campylobacter culture-positive patient during the acute phase of illness (4 to 8 days postonset) and during convalescence (11 to 18 days postonset). Control urine specimens were obtained from healthy Indonesian children of approximately the same age. Species identification of Campylobacter isolates was based on growth at 42°C, hippurate hydrolysis, and susceptibility to nalidixic acid and cephalothin. Isolates were serotyped by the slide agglutination technique (4).

Western blotting was done with protein preparations of Campylobacter jejuni and C. coli. Cells were sonicated and electrophoresed on sodium dodecyl sulfate-polyacrylamide gels as described previously (9). Protein concentration was determined by the Bio-Rad (Richmond, Calif.) protein assay. After electrophoresis, proteins were transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, Mass.) and immunostained as described previously (9). A chemiluminescent substrate was compared with chromogenic substrates for sensitivity in a Western blot assay. Limited quantities of urine did not permit both IgA antibody titer determinations and comparisons of substrates. Therefore, evaluations of substrates were performed with serial dilutions of C. coli and mouse anti-C. coli serum. Strips with antigens in triplicate were incubated with mouse anti-C. coli serum and then peroxidase-conjugated goat anti-mouse IgG and IgM and developed with one of three different substrates: tetramethylbenzidine (TMB; Kirkegaard & Perry) for 3 min, 4-chloro-1-naphthol (Kirkegaard & Perry) for 20 min, or enhanced chemiluminescence (ECL) reagents (Amersham, Arlington Heights, Ill.) for 1 min. The ECL strips were exposed to X-Omat AR films (Kodak) for 10 s, and the films were developed in a Kodak X-Omat M20 Processor. The TMB and 4-chloro-1-naphthol membrane strips and films for ECL were scanned by UltraScan XL laser densitometer (Pharmacia LKB, Piscataway, N.J.).

For titration of urine by ECL, antigen strips were incubated with serial twofold dilutions of a urine specimen and then incubated in a 1:1,000 dilution of peroxidase-conjugated goat anti-human IgA (Kirkegaard & Perry) prior to detection by ECL. A specimen was considered positive for Campylobacter-specific IgA by demonstrating binding of a 1:2 or greater dilution of urine to all three common Campylobacter antigens (18, 43, and 62 kDa). Titters were expressed as the reciprocal of the highest dilution of urine that yielded reactivity.

All of the six Campylobacter isolates were identified as C. jejuni, but only three were of an identifiable Lior serotype (Table 1). Urinary IgA specific for three common antigens of Campylobacter spp. (18, 43, and 62 kDa) was detected by Western blotting of acute-phase specimens from all Campylobacter-positive patients (Fig. 1). Controls and convalescent-phase specimens were negative. The 43- and 62-kDa antigens may represent the major outer membrane protein and immune dominant flagellar protein, respectively. The molecular weights of these antigens were similar to those reported previously (1, 6). The 18-kDa antigen (Fig. 1) may be related to the 14.5-kDa antigen detected in stools of Campylobacter-infected patients (8).

The Campylobacter-specific IgA titers in this assay ranged from 2 to 8 (Table 1). The highest level of IgA was detected on day 4 postonset, and by least-squares regression, titers were estimated to decrease to undetectable levels by day 9.

* Corresponding author.
TABLE 1. Campylobacter-specific urinary IgA titers of six children with Campylobacter-associated diarrhea and three healthy children, determined by Western blotting

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Clinical status</th>
<th>Sex</th>
<th>Age (mo)</th>
<th>Presence of the following clinical sign:</th>
<th>No. of stools in previous $24 \text{ h}$</th>
<th>C. jejuni serotype</th>
<th>IgA titer in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fever</td>
<td>Watery stool</td>
<td>Blood in stool</td>
<td>Mucus in stool</td>
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<tr>
<td>1</td>
<td>Diarrheic</td>
<td>M</td>
<td>7</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Diarrheic</td>
<td>F</td>
<td>15</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Diarrheic</td>
<td>M</td>
<td>19</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Diarrheic</td>
<td>M</td>
<td>26</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Diarrheic</td>
<td>F</td>
<td>11</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>Diarrheic</td>
<td>M</td>
<td>7</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>Healthy</td>
<td>M</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>Healthy</td>
<td>F</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>Healthy</td>
<td>M</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ M, male; F, female.
$^b$ Determined by the serotyping method of Lior.
$^c$ NA, not applicable.
$^d$ NS, not serotypeable.
$^e$ NR, not recorded.

The successful application of this assay to multiple serotypes and the likely similar prevalence of these serotypes in all age groups indicate that this immunodetection system may be useful for the diagnosis of a substantial proportion of Campylobacter infections that occur worldwide. This is the first demonstration by the Western blot assay of Campylobacter-specific IgA in human urine specimens. Although our findings are based on preliminary studies and do not provide enough information to assess the Western blot technique fully as a diagnostic tool, the data suggest that (i) a single-determination test result (with a titer of 2 or greater) early in the illness may provide a rapid preliminary diagnosis for young children, (ii) the window for use of this test in young patients. Previous enzyme-linked immunosorbent assay-based estimates of Campylobacter IgA titers in urine specimens from adult patients in the United States indicated that titers peaked at 11 to 15 days postonset, with a rapid decrease over the next 1 to 5 days (3). The finding that Campylobacter-specific IgA developed earlier and decreased more rapidly in Indonesian children than in American adults (3) may be due in part to the relative likelihood of previous exposure of the Indonesian children.

The ECL indicator was about 10-fold more sensitive than 4-chloro-1-naphthol; however, it was as sensitive as TMB, on the basis of Western blot patterns (Fig. 2) and densitometer values (data not shown). The ECL indicator provided a more intense, nonfading stain than the colored precipitate produced by TMB.

**FIG. 1.** Demonstration of Campylobacter-specific IgA in human urine specimens by Western blot assay. Membrane strips with electrophoresed C. coli (lane A) and C. jejuni (lane B) were incubated with human urine (patient 1). Bound Campylobacter-specific IgA was detected with peroxidase-conjugated goat antihuman IgA and ECL detection reagents.

**FIG. 2.** Comparison of Western blots employing either chemiluminescent or chromogenic substrates as an indicator for demonstrating specific mouse antibody to C. coli. Lanes: A, ECL, 1 min, exposed to X-ray film for 10 s; B, TMB, 3 min; C, 4-chloro-1-naphthol, 20 min.
children is 1 week postonset of diarrhea, and (iii) since the highest antibody titer detected was on day 4 postonset (the first day urine samples were available in this study), detectable levels of urinary IgA are probably present even earlier. Further investigation is in progress to assess the sensitivity and specificity of the assay and its further adaptation to a rapid flowthrough membrane assay format.

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