Campylobacter jejuni Enteritis

Shahida Baqar,1 Brandt Rice,1 Lanfong Lee,1 A. Louis Bourgeois,1 Amina Noor El Din,2 David R. Tribble,1 Gloria P. Heresi,3,4 Aly S. Mourad,4a and James R. Murphy4a

1United States Naval Medical Research Center, Silver Spring, Maryland; 2Department of Bacteriology, Alexandria University, Alexandria, and 3Division of Pediatric Infectious Diseases, University of Texas–Houston Medical School of Defense, or the US government. Not to be construed as official or as reflecting the view of the Navy Department, Department of the Army, Department of the Air Force, or the US government.

We report the development of Campylobacter jejuni enteritis in a patient with preexisting humoral and cellular immune recognition of C. jejuni antigens. This is one of few studies in which the immunologic status of a person with regard to C. jejuni before and after C. jejuni infection is directly compared, and it is the only study of which we are aware that includes measurements of cellular immunity. The findings may be important to Campylobacter vaccine development efforts.

Campylobacter jejuni is a common cause of enteritis worldwide [1]. Contact with Campylobacter is frequent, and ~1% of the population acquires Campylobacter infection per year in the United States and Britain [2]. Much higher rates occur in less-developed countries and among travelers to these regions; an estimated 27,000–38,000 infections occur per 100,000 travelers to less-developed countries per year [1, 2]. Rates of acquired immune recognition of C. jejuni rise rapidly with increasing age [3, 4], and, in areas of high C. jejuni endemicity, the frequency of C. jejuni disease decreases with increasing age [4]. Studies of induced C. jejuni infection of volunteers demonstrated that infection resulted in production of antibodies specific for C. jejuni antigens and in protection from illness after a second challenge with the same organism [5]. The morbidity associated with Campylobacter infections and the evidence for immune protection against C. jejuni disease are the foundation of ongoing efforts to formulate vaccines against C. jejuni [4]. However, vaccine construction is confounded by the lack of knowledge of virulence mechanisms and the antigenic targets of protective immune responses and by the knowledge that adults who have had Campylobacter infection may develop subsequent enteritis caused by Campylobacter [6]. The serendipitous natural acquisition of C. jejuni infection by a volunteer participant in ongoing studies for whom an archive of plasma and mononuclear cells existed enabled a study of this patient’s immune recognition of C. jejuni before, during, and after the current infection.

Case report. A previously healthy 46-year-old man, a US expatriate residing in Egypt, developed fever (temperature, 40°C), headache, and myalgia while en route from Cairo to the United States (case day 0). The patient had lived in Egypt for the past 4 years and had not traveled outside of Egypt during the preceding month. Watery diarrhea developed the next morning, and stool frequency peaked on the third day of illness, at 14 watery stools per day. Fever, headache, and myalgia persisted. The patient presented to a local hospital on the third day of illness. Physical examination revealed a temperature of 38.9°C, a pulse rate of 109 beats/min, and abdominal tender-
ness. Laboratory findings included the following: negative blood cultures, WBC count of 7000 cells/mm³, fecal leukocytes 4+, negative results of Hemoccult (Beckman Coulter) testing of stool, and negative results of testing of stool for ova or parasites. Treatment with ciprofloxacin (500 mg twice daily) was initiated, and symptoms and signs waned and disappeared completely by day 5.

On day 6, the laboratory reported the isolation of C. jejuni from a day 3 stool sample. On day 7, treatment was changed to erythromycin (250 mg 4 times a day for 5 days). Results of testing of a second stool sample collected at day 8 and evaluated at the US Naval Medical Research Center were negative for C. jejuni and positive for gross blood; showed WBCs in sheets; and were positive for lactoferrin, through a dilution of 1:1 million by a latex agglutination test (TechLab). Additional blood samples were positive for lactoferrin, through a dilution of 1:1 million by a latex agglutination test (TechLab). Additional blood samples were sent for testing for C. jejuni.

The isolate (designated “isolate 20-01”) was obtained from the hospital. Tests made at the Naval Medical Research Center confirmed that the bacterium was C. jejuni. Gram stain and scanning electron microscopy (see figure 136-1 in Heresi and Cleary [2]) revealed staining and morphology consistent with a flagellated C. jejuni. Selected tests of phenotypic characteristics showed a pattern consistent with C. jejuni: the isolate grew on Campylobacter blood agar (Remel) and was catalase positive, hippurate positive, nitrate reduction positive, urease negative, and hydrogen sulfide negative; further, it was sensitive to nal- idixic acid, resistant to cephalothin, had no growth at 25°C, and showed growth at 42°C. Silver-stained sodium dodecyl sulfate–PAGE gels revealed a protein profile for 20-01 that was similar to that of reference strain 81-176 (figure 1). The case isolate did not react in the Lior serotyping schema, in which C. jejuni 81-176 yielded a result of type 5 (testing for 120 serotypes was done at the Laboratory Center for Disease Control [Ottawa]), and 20-01 had a slightly higher virulence for BALB/c mice than the well-studied 81-176 isolate [8].

**Immunologic recognition of C. jejuni before onset of symptoms.** Table 1 presents patterns of immunologic recognition of isolates 20-01 and 81-176 seen in plasma and mononuclear cells collected 801 and 261 days before onset of the illness currently under discussion. Most serologic procedures were applied to plasma samples collected on day −801 and on day −261. Results at the beginning and end of this interval were remarkably similar and have been averaged. Antibodies reacting with C. jejuni 20-01 and 81-176 were found. Immunoblots made on plasma collected at −261 days showed IgG antibodies that reacted with numerous C. jejuni antigens (figure 2, panel −261, rows 2 and 3); antigens prepared from isolates 20-01 and 81-176 were similarly recognized. IgA reacted with fewer antigens, but the different C. jejuni isolates were recognized nonetheless.

Tests of mononuclear cell immune recognition of C. jejuni were made on cells obtained at day −261 (table 1). The patient did not have antibody-secreting cells (ASCs) that reacted with any of the tested C. jejuni antigens. In contrast, mononuclear cells tested by antigen-driven lymphocyte replication assay showed notable recognition of isolate 81-176, but not 20-01, antigen. The volunteer had humoral and cellular immune recognition of C. jejuni before the current infection.

**Responses to C. jejuni infection.** Rapid, vigorous, and sustained antibody responses specific to C. jejuni were seen after infection (figure 3). Marked increases in anti-C. jejuni antibodies were present at day 3, most responses peaked at day 10,
Rapid and vigorous ASC responses specific to *C. jejuni* were documented (figure 4). Significant increases in *C. jejuni*–specific ASCs were present at day 3, and IgM responses peaked at this time, whereas IgA and IgG ASC responses peaked at day 10. All responses waned to near undetectable levels by day 34. ASC responses were both genus and isolate specific. The IgA and

and all responses persisted through day 120. Antibody responses had both genus and species specificity and a degree of isolate specificity. Responses to isolate 20-01 were somewhat more vigorous, more rapid, or both than responses to 81-176. The antibody response to 81-176 lipopolysaccharide was weak and delayed compared with the other responses, and it lacked an IgA component. Western blot analyses showed rapid and sustained increases in antibodies recognizing multiple *C. jejuni* components (figure 2). Both the numbers of antigens detected and the density of bands increased by day 3 and peaked at day 10 (IgA) or 34 (IgG). Enhanced IgG recognition persisted at least through day 120, whereas IgA responses waned substantially by day 34. Although the majority of antigens detected were shared between the 2 strains, 20-01 had 2 bands (at 34 and 31.5 kDa) that were unique.
Figure 4. Cellular immune responses. Mononuclear cells were isolated from blood samples collected at each interval and cryopreserved. For each assay, cells from each bleeding interval were recovered from the freezer and tested concomitantly by means of established methods [8, 9, 11]. Results of antibody-secreting cell assays are presented as no. of antibody-secreting cells of an isotype specific for the indicated antigen in a population of $10^6$ peripheral blood mononuclear cells. Data for antigen-driven lymphocyte replication assays are presented as change in mean counts/min (CPM), calculated as follows: (geometric mean of triplicate cultures of cells collected at an interval after infection) / (geometric mean response to the same antigen before infection). This presentation emphasizes the relative changes in recognition of antigens prepared from homologous and heterologous Campylobacter isolates (geometric mean, backtransformed). CPM values were for day -261 samples: 142, 143, 1118, 370, and 1121 for cultures without antigen or containing Campylobacter jejuni isolate 20-01, C. jejuni isolate 81-176, Campylobacter fetus, or Escherichia coli antigens, respectively. LPS, lipopolysaccharide.

IgG ASC responses to 81-176 glycine extract antigen were approximately 50% of responses to isolate 20-01 glycine extract antigen.

Lymphocyte replication assays (figure 4) showed a rapid, vigorous, sustained, and specific response to C. jejuni 20-01 antigen. Increased response was seen at day 3, and this persisted at increased levels at least through day 120. In contrast, there was little change in lymphocyte replication response to 81-176 antigen as the result of infection.

Cytokine and C-reactive protein (CRP) responses were rapid (figure 5). With the exception of TNF-$\alpha$ levels, which were never elevated, plasma inflammatory cytokines and CRP levels were increased on day 3. IL-8 levels peaked at day 3 and IL-1 and CRP levels on day 10. In contrast, regulatory cytokines (IL-6 and IFN-$\gamma$) were not detected at day 3 but were present in high concentrations at day 10.

Discussion. This case of C. jejuni enteritis occurred in a man whose preinfection immunologic markers denoted previous experience with Campylobacter. The occurrence of disease shows that this immunity was not protective. Because there was no evidence of immune deficit before or after this illness, it is likely that the illness resulted from infection either with a Campylobacter capable of defeating the existing immune mechanisms or with a C. jejuni that differed in antigenic targets of protective immune responses from C. jejuni previously encountered by the patient. Direct evidence was obtained that the causative bacterium was antigenically special. Some notable differences were seen: in essentially all immunologic assays, responses to 20-01 were more rapid or greater than responses to 81-176; plasma collected after infection uniquely recognized 31.5- and 34-kDa proteins in 20-01; and 20-01 was untypeable by the Lior serotyping system. The 31.5-kDa protein is consistent with the known mass of PEB4 [7], a surface component that is known to vary markedly among Campylobacter isolates. The identification of the 34-kDa unique protein is not known. On the other hand, numerous antigens were shared between isolates 20-01 and 81-176, and immune recognition of some of these predated the current infection. Notable in this regard were strong antibody responses to an $\sim$60-kDa protein that may correspond to flagellin [12]. This protein was similarly recognized in 20-01 and 81-176 antigen preparations and was strongly recognized by plasma collected before the present infection. The significance of this observation to flagellin-based vaccine development deserves consideration.

Western blot analyses made as part of this study differ from those reported previously; an increased number of antigens and an increased density of bands were detected in plasma collected before infection. The pattern of antigens detected by Western blots in after-infection plasma is, in general, similar to those previously reported [13–16]. It is possible that the 20-01–specific 31.5- and 34-kDa proteins are similar to patient isolate–specific
proteins of approximately these masses visualized by Mills and Bradbury [16] in serum samples from a laboratory worker infected with a known C. jejuni strain.

It is notable that the subject of this study lacked lymphocyte response to isolate 20-01 before the present infection, yet developed this as a result of the infection. In contrast, the man had lymphocyte response to 81-176 before and after infection, and lymphocyte response to 81-176 was not boosted by 20-01 infection. Because the antigen used to elicit these responses preferentially detects immune recognition of bacterial surface components [11], the results indicate that 20-01 is antigenically different from 81-176. This suggests that cellular immune response to a C. jejuni surface component may correlate with protection from disease.

It was also notable that acute and resolved infections were 4-fold higher than the day 3 level. The ASC procedure was best for making an immunologic diagnosis of infection, a finding consistent with studies of other enteric bacterial pathogens [17].

C. jejuni strain 20-01 was capable of causing illness in a man in the face of established immune recognition of C. jejuni; the strain differs antigenically from 81-176, a strain that was used in previous and ongoing volunteer studies. The 20-01 strain may be useful in studies to resolve protective immune responses and mechanisms of pathogenesis.

Figure 5. Selected cytokines and C-reactive protein (CRP). Commercial kits were used (Intergen; RapiTex CRP, Behring).

References