An Unusual Case of Refractory Campylobacter jejuni Infection in a Patient with X-Linked Agammaglobulinemia: Successful Combined Therapy with Maternal Plasma and Ciprofloxacin

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An unusual hippurate-negative strain of Campylobacter jejuni caused a chronic refractory infection in a patient with X-linked agammaglobulinemia; this infection persisted for >2 years despite therapy with various antibiotics and immunoglobulins (Igs). To characterize the defense status of this patient, several in vitro studies, including those with T cells and polymorphonuclear leukocytes (PMNLs), were performed. T cell responses specific for C. jejuni were only weak in this patient. Chemiluminescence and bacterial killing studies with PMNLs revealed that the bactericidal activity of PMNLs against Campylobacter was enhanced more vigorously by maternal serum than by commercial Ig preparations. On the basis of these results, combined treatment with ciprofloxacin and maternal plasma was initiated, and the C. jejuni infection was rapidly cured. This case report shows that in vitro immunologic assays may be useful for characterizing immune functions of patients with chronic or refractory C. jejuni infections, thus leading to individual treatment strategies.

Case Report and Laboratory Investigations

A baby boy was born as the third child of healthy parents. At 6 months of age, severe hypogammaglobulinemia was diagnosed; the count of circulating B cells was very low, but the counts and functions of T cells were normal. Since his older brother also had extremely low serum levels of Ig as well as no circulating B cells, XLA was diagnosed and confirmed by indirect DNA methods (including restriction fragment length polymorphism analysis). The boy received regular intravenous infusions of Ig (Endobulin containing pooled human Igs [99.9%, IgG; 0.1%, IgA], Immuno, Heidelberg, Germany) at a dose of 400 mg/kg of body weight every 4 weeks and was doing well except for repeated episodes of otitis media.

At the age of 7 years, the boy was admitted to the Children's Hospital of Würzburg Medical School (Würzburg, Germany) because of a 4-month history of a generalized pruritic rash. Physical examination revealed numerous grouped and disseminated maculopapular skin lesions with small vesicles or crusts on the summit (figure 1). There was extensive excoriation. The lesions were most pronounced on the trunk and on the extensor surfaces of the extremities. There were no other abnormal physical findings. The patient was afebrile and had no history of fever or chills during the preceding months.

Laboratory investigations revealed an erythrocyte sedimentation rate of 4 mm/h and a WBC count of 12.6 × 10^9/L (57% neutrophils, 25% lymphocytes, 11% monocytes, 4% band forms, 2% eosinophils, and 1% basophils). Three weeks after the last dose of Endobulin, the serum concentration of IgG was 475 mg/dL. IgA, IgM, and IgE levels were undetectable. Total complement activity (CH50, AP50) was normal.
After admission, routine procedures were used to isolate \textit{C. jejuni} several times from blood and stool specimens from the patient; cultures of the samples on plates of meat-yeast extract agar were incubated for 48 hours at 42°C under microaerobic conditions (Anaerocult C, Merck, Darmstadt, Germany). The strain did not hydrolyze hippurate but was identified as \textit{C. jejuni} by determination of 16S rRNA gene sequences according to the method of Giesendorf et al. [11] and as \textit{C. jejuni} serotype Lior 11 by slide agglutination according to the method of Lior et al. [12]. A region of the 16S RNA gene was amplified by PCR analysis with the \textit{Campylobacter}-specific primer pair C422 and C490, which correspond to \textit{Escherichia coli} positions 399–420 and 825–803, respectively [12]. The resulting PCR fragment (426 bp) was sequenced by the Taq-cycle Dye Deoxy terminator method [13] in combination with a 373A automatic sequencer (Perkin Elmer, Weiterstadt, Germany). Database searches revealed 100% sequence homology with \textit{C. jejuni}. The presence of polymerase inhibitory substances and the specificity were controlled with the use of human β-actin-specific primers [13], which amplified a fragment of 348 bp.

The \textit{C. jejuni} strains repeatedly isolated from the patient before and after antimicrobial therapy appeared to be identical (determined by the above-mentioned methods) and exhibited an identical pattern of antimicrobial susceptibility, thus suggesting chronic persistent infection rather than reinfection with other strains of \textit{C. jejuni}. The strains were susceptible to gentamicin (10 μg), tetracycline (30 μg), imipenem (10 μg), and ciprofloxacin (5 μg) but were resistant to erythromycin (15 μg), ampicillin (10 μg), mezlocillin (30 μg), cefotiam (30 μg), cefotaxime (30 μg), and co-trimoxazole (25 μg).

The boy was initially treated intravenously with amoxicillin (500 mg three times a day for 14 days) and doxycycline (200 mg/d for 10 days) and then with imipenem (500 mg twice a day for 10 days). He also received bovine colostrum (10 g orally twice a day for 10 days; Lactobin, Biotest Pharma, Dreieich, Germany). Moreover, Endobulin was substituted by an intravenous IgM-containing preparation (Pentaglobin,

\textbf{Figure 1.} \textit{Campylobacter jejuni}–induced skin lesions in a patient with X-linked agammaglobulinemia before (left) and 2 months after (right) combined treatment with ciprofloxacin and maternal plasma.
Biotest Pharma). Although blood cultures became sterile, serial stool cultures, spanning nearly 18 months, demonstrated persistent excretion of C. jejuni. Furthermore, there was no beneficial effect of treatment on skin manifestations, which appeared to be intractable.

Several months later, skin biopsy specimens were taken to elucidate the etiology of the skin alterations. Although skin aspirates and biopsy specimens were sterile, PCR techniques revealed the presence of C. jejuni DNA in the skin lesions, thereby suggesting that the skin lesions were due to C. jejuni infection (figure 2). Cultures of parallel blood specimens taken at this time were negative.

Therefore, on the basis of the results obtained by the in vitro studies (which are discussed below) and with the informed consent of both parents, the decision was made to treat the boy with maternal plasma (10 mL/kg of body weight·w) for 5 weeks in combination with ciprofloxacin (250 mg orally twice a day for 3 weeks). Stool cultures became negative 5 days after the start of treatment, and the skin lesions completely resolved within 6 weeks (figure 1). During a 12-month follow-up period, stool cultures remained negative, and the patient continues to be asymptomatic.

Experimental Procedures

Proliferative responses of peripheral blood mononuclear cells (PBMCs). PBMCs were isolated by Ficoll (Biochrom, Berlin) density gradient centrifugation. PBMCs (10^5) were inoculated in triplicate into wells on 96-well microtiter plates that contained RPMI 1640 tissue culture medium (Biochrom, Berlin) supplemented with 10% AB serum (Sigma, Deisenhofen, Germany) and horse serum (Gibco BRL, Eggenstein, Germany). The cells were stimulated with 10 μg of a heat-killed preparation of C. jejuni, Listeria monocytogenes, Yersinia enterocolitica, or Salmonella typhimurium per milliliter or with 100 μg of tetanus toxoid per milliliter. Six days later, 0.5 μCi of [3H]thymidine was added to each well. After a further 6 hours of incubation, cells were harvested, and [3H]thymidine uptake was determined.

PMNL functions. Blood was taken from the patient, his mother, and unrelated healthy controls (laboratory personnel). PMNLs were prepared as recently described [14]. Luminol (Sigma)-amplified chemiluminescence (CL) of PMNLs was performed, and the PMNL responses were measured by a microplate chemiluminometer (Hamamatsu Photonics, Herrsching, Germany) at 37°C as described previously [14]. Briefly, 10^5 PMNLs per well were incubated with RPMI 1640 cell culture medium containing 2.5 × 10^{-5} M luminol. For stimulation, viable C. jejuni (10–100 bacteria per PMNL) or 10 μg of heat-killed C. jejuni or zymosan (Sigma, Munich) was added to various Ig preparations or sera. Light emission was recorded continuously for 90 minutes. The total light emission was calculated as previously described [14]. Three separate experiments with triplicate wells revealed comparable results.

Modulation of the bactericidal activity of PMNLs against C. jejuni was shown by the above-described microplate assay with various Ig preparations and sera. PMNLs were incubated with C. jejuni at a ratio of 1:100 for 30 minutes at 37°C. Subsequently, 30 μg of gentamicin/mL was added to kill extracellular bacteria. Two hours later, cells were washed and lysed with PBS containing 0.5% bovine serum albumin and 0.5% Tergitol (Fluka, Buchs, Switzerland).
Duplicates of serial dilutions of these suspensions were plated on meat-yeast extract agar, and cfu were counted after incubation for 48 hours at 42°C.

Statistical analysis. Differences between mean values were analyzed by the Student’s t test. P values of <.05 were considered statistically significant.

Results and Discussion

A hippurate-negative C. jejuni strain caused a chronic refractory infection in a patient with XLA; this infection persisted despite treatment with different antibiotics (amoxicillin, doxycycline, and imipenem) and intravenously and orally administered Ig preparations (Endobulin, Pentaglobin, and Lactobin). This treatment caused clearance of C. jejuni from the blood but not from the intestine and skin lesions.

The rationale for new treatment with maternal plasma and ciprofloxacin for this patient was based on the findings of PCR analysis, which revealed that C. jejuni may persist not only in the intestine (or the blood) but also in tissue (e.g., skin) lesions. Moreover, the possibility that C. jejuni may persist intracellularly cannot be excluded, and thus, this persistence may evade substitution therapy with Ig or certain antibiotics (such as gentamicin) that only act extracellularly. Therefore, we believed that combination therapy with antibiotics that act both intracellularly and extracellularly and immunomodulating compounds that act rather extracellularly should mediate a rapid clearance of the bacteria from both the blood and the gut; on the other hand, we thought that this combination therapy should stimulate and support the defense status of this patient by eliminating the pathogen at sites where antibiotics were not effective (e.g., skin abscesses).

Moreover, antibiotic therapy alone, in the absence of host defense mechanisms, often appears to be unable to cure infections, especially those with intracellular pathogens (authors’ unpublished observations). Further, a previous study [2] reported the beneficial effect of combination therapy with imipenem and plasma for chronic campylobacter infections.

Before the initiation of the new treatment, in vitro studies were performed to investigate whether the patient’s PMNLs were principally able to kill the pathogen at least under experimental conditions and which immunologic compound might be effective as immunomodulating treatment. To evaluate the bactericidal activity of the patient’s PMNLs against Campylobacter, CL responses of PMNLs were assayed in the presence of various Ig preparations and sera. The patient’s PMNLs showed only weak CL responses when they were exposed to viable C. jejuni in the patient’s serum or commercial Ig preparations (figure 3). Experiments with heat-killed C. jejuni revealed comparable results (data not shown).

In agreement with recently published results [10], only IgM-containing preparations (Pentaglobin) enhanced the bactericidal effects of PMNLs against Campylobacter (P < .05). However, the strongest responses to CL were observed when PMNLs were exposed to C. jejuni in serum derived from the patient’s healthy mother (P < .05; figure 3). Likewise, comparable results were obtained when the mother’s PMNLs (P < .05) or PMNLs from unrelated healthy controls (data not shown) were used (figure 3), thus suggesting that certain components of the patient’s serum that are required for triggering C. jejuni-specific CL responses by PMNLs were lacking. In addition, stimulation with zymosan, which is opsonized by complement C3b, caused comparable CL responses by PMNLs from the patient and his mother (figure 3), thereby suggesting that C. jejuni-specific compounds (such as antibodies) were lacking in the patient’s serum.

Since it has not yet been established that reactive oxygen metabolites mediate the killing of C. jejuni, the bactericidal activity of PMNLs was directly determined. For this purpose, PMNLs were mixed with viable C. jejuni in various sera and Ig compounds, and a gentamicin assay was performed. The results (figure 4) showed that the CL responses of PMNLs that are depicted in figure 3 correlated directly with bacterial killing by PMNLs. Thus, apart from Pentaglobin, which significantly enhanced the bactericidal effects of PMNLs against C. jejuni (P < .05), the commercial Ig preparations had no effect and even reduced killing. However, as observed for CL responses, addition of maternal serum to the patient’s PMNLs most vigorously enhanced the bactericidal activity against Campylobacter (P < .05).

On the basis of these results, the decision was made to transfuse maternal plasma to enhance the patient’s defense against C. jejuni infection. Since the patient’s clinical condition was worse...
Therapy with ciprofloxacin (250 mg orally twice a day) was continued for another 20 days. Five days after the therapy was started, *C. jejuni* could no longer be detected in blood, skin, and stool specimens, thus suggesting that the combination therapy cured the *C. jejuni* infection in this patient. Follow-up for a further 12 months confirmed that the chronic refractory *C. jejuni* infection in this patient was cured with combination treatment with maternal plasma and ciprofloxacin.

However, we do not yet know which component of maternal plasma mediated the observed immunobiological effects. Surprisingly, we detected only insignificant low levels of *C. jejuni*-specific antibody in maternal serum by means of immunoblotting (data not shown). Moreover, it is not yet known whether inhibitory components were present in the patient’s serum as suggested by the data presented in figures 3 and 4. Thus, systemic studies are required to elucidate the therapeutic effect of maternal plasma. On the other hand, we cannot exclude the possibility that ciprofloxacin therapy alone might have caused eradication of the pathogen.

It is interesting that T cell responses specific for *C. jejuni* in our patient were very weak (*P < .05*) only when they were compared with those in a patient with acute *C. jejuni* infection, while T cell responses upon exposure to a recall antigen (tetanus toxoid) in our patient were pronounced compared with those in controls (table 1). Whether these findings are because of a so far unknown T cell deficiency in the patient, a T cell tolerance due to persistent infection, or a deficiency in antigen presentation as a result of the lack of phagocytosis of *C. jejuni* in vivo is not yet clear. However, although the role of T cells in the host response to *C. jejuni* infection has yet to be established, recent data from models of infection with other enteroinvasive, extracellular bacteria (such as *Y. enterocolitica*) argue for such a role [15, 16].

Finally, the strain isolated from our patient did not hydrolyze hippurate but was identified as *C. jejuni* by 16S rRNA gene sequencing. Although >99% of *C. jejuni* strains do hydrolyze...
hippurate while *Campylobacter coli* strains do not, we and other investigators [17] recommend that hippurate hydrolysis [18] should not be used as a sole criterion for differentiation between these *Campylobacter* species.

References