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Persistence of *Campylobacter fetus* Bacteremia Associated with Absence of Opsonizing Antibodies

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***Campylobacter fetus* causes systemic infections in immunocompromised hosts. We describe a case in which *C. fetus* bacteremia apparently relapsed after 7 years in a patient with hypogammaglobulinemia and characterize the serum resistance of the patient's *C. fetus* strain and the inability of the patient's serum, with and without commercial intravenous immunoglobulin, to opsonize this and another *C. fetus* strain effectively. The probable presence of a sequestered site of infection in bone, the intrinsic serum resistance of the *C. fetus* strain, and the absence of specific antibody may account for the persistent infection in this patient. These studies suggest that intravenous immunoglobulin treatment is not useful in eradicating *C. fetus* bacteremia.**

Campylobacter fetus subsp. *fetus* is an infrequent human pathogen that causes bacteremia and a wide range of systemic illnesses in immunocompromised hosts (4). Systemic infection is most frequently diagnosed in men over the age of 50 years with underlying disease, especially chronic liver disease, malignancy, or hematologic disorders (4). *C. fetus* has a remarkable ability to cause prolonged or relapsing bacteremia without evidence of endocarditis (9, 10), but the basis for this phenomenon has not been well explored. Regardless of the host, *C. fetus* strains resist complement-mediated killing by normal human serum and phagocytosis by neutrophils (2, 3). This resistance of *C. fetus* to C3 binding is due to the presence of surface array proteins (S-layer proteins) which encapsulate the organism (1, 3, 11).

We present a case of previously treated *C. fetus* bacteremia that apparently relapsed after a disease-free interval of 7 years in a patient with pure red cell aplasia, malignant thymoma, and acquired hypogammaglobulinemia. This case represents the longest reported dormancy of *C. fetus*. The pathogenesis of this patient's infection was studied by characterizing the resistance of his *C. fetus* isolate to the opsonic activity of selected sera, including his own. The potential opsonizing role of intravenous (i.v.) immunoglobulin (Ig) in treatment was also examined.

CASE REPORT

A 50-year-old man was diagnosed with pure red cell aplasia and malignant thymoma in June 1985. The thymoma was resected through a median sternotomy approach in July 1985, followed by radiation therapy. Ten days postoperatively, the patient developed fever without gastrointestinal symptoms, and blood cultures yielded *C. fetus* subsp. *fetus*. He received 2 weeks of i.v. erythromycin, followed by 2 weeks of oral erythromycin, and follow-up blood cultures were negative. The patient did well until October 1990, when he developed sinusitis. Over the next 2 years, he had recurrent bouts of bronchitis and sinusitis, requiring multiple courses of antibiotics and two sinus surgeries. In October 1992, he was found to have decreased Ig levels in his serum: IgG, 70 mg/dl (normal, 639 to 1,349 mg/dl); IgA, 5 mg/dl (normal, 70 to 312 mg/dl); and IgM, 5 mg/dl (normal, 56 to 352 mg/dl). On the basis of the triad of acquired hypogammaglobulinemia, prior thymoma, and aplastic anemia, the diagnosis of Good's syndrome was made (7). At that time, the patient received a course of ofloxacin and corticosteroids for a severe episode of sinusitis. On day 23 of antibiotic therapy, he developed tenderness, swelling, and erythema of the right clavicular head. He was admitted to Vanderbilt University Hospital, where a bone scan showed increased activity in the right clavicular head, consistent with osteomyelitis. A bone marrow aspirate from the iliac crest showed decreased plasma cells. He received 5 days of i.v. g and 4 weeks of imipenem (500 mg i.v. every 6 h), followed by 3 weeks of ciprofloxacin (750 mg orally every 12 h). One of two blood cultures drawn 5 days after the antibiotics were stopped grew *C. fetus* subsp. *fetus*, and ciprofloxacin was resumed. MICs were 4.0 μg of ciprofloxacin per ml, <0.12 μg of azithromycin per ml, and 0.06 μg of imipenem per ml. Treatment was changed to 250 mg of azithromycin given orally every 12 h, but 3 weeks later, while therapy was still being given, a blood culture again yielded *C. fetus* subsp. *fetus*. The patient was readmitted to Vanderbilt University Hospital, where a transesophageal echocardiogram was normal, a bone scan showed increased activity in the right clavicular head, but less so than the previous study, and an abdominal ultrasound test showed gallstones. Imipenem treatment was restarted. Although the patient had no gastrointestinal complaints, the gallbladder was considered a possible source of persistent infection. He underwent laparoscopic cholecystectomy, and the pathologic examination showed gallstones and chronic inflammation, but all cultures were negative. He continued to receive periodic i.v. Ig therapy based on Ig levels in serum. Weekly surveillance blood cultures during a 4-week course of imipenem were negative. Three days after the imipenem was discontinued, the patient had fever, malaise, and myalgias. Blood cultures again yielded *C. fetus* subsp. *fetus*. On readmission to Vanderbilt University Hospital in April 1993, physical examination revealed a temperature of 101°F (about 39°C), an area (4 by 4 cm) of erythema at the medial edge of the cholecystectomy scar, and tenderness of the underlying ribs to palpation. Blood cultures

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again grew *C. fetus* subsp. *fetus*. On imipenem therapy, the patient defervesced and his cellulitis improved. A repeat transesophageal echocardiogram was normal, and a repeat bone scan showed persistent activity in the right clavicle and a new area of increased activity in the chondral cartilage overlying the right upper quadrant. Computerized tomography of the chest showed no evidence of recurrent thymoma. Since the patient appeared to have multiple foci of infection, it was decided to treat him for 4 more weeks with imipenem, followed by lifelong oral suppression with 500 mg of clarithromycin taken orally every 12 h. After 10 months of clarithromycin, the patient continues to be asymptomatic and blood cultures remain negative.

MATERIALS AND METHODS

Wild-type strains of *C. fetus* are usually serum resistant (3, 10) but can be opsonized by specific antibodies for uptake and killing by phagocytic cells (3). To determine whether the patient's isolate was serum and phagocytosis resistant, serum bactericidal and opsonophagocytosis assays were performed as previously described (3). The patient's *C. fetus* strain (strain 7307), another wild-type *C. fetus* strain known to possess the S-layer protein (strain 23D), and a mutant strain that lacks the S-layer protein (strain 23B) (1, 3, 11) were studied. Serum bactericidal assays using pooled normal human serum were performed exactly as previously described (2). For opsonophagocytosis assays, inocula of 1,000 to 3,000 *C. fetus* cells in 10 μl of Hanks balanced salt solution were incubated for 60 min with 170 μl of whole blood (phagocytic cell source) from a healthy donor with no history of *C. fetus* infection and with 20 μl of pooled normal human serum (as an antibody source for opsonization). Serial dilutions of initial and 60-min samples were cultured to determine viable cell counts, and results from two to four runs were analyzed. As a negative control, parallel experiments were performed with normal human serum that was heat inactivated (HI) at 56°C for 30 min to eliminate complement activity. We also tested immune rabbit serum raised against *C. fetus* S-layer proteins, two commercially available i.v. Ig preparations (5% Gamimune-N [lot 40X147; Miles Inc., Elkhart, Ind.] and 5% Venoglobulin [lot GL3520A; Alpha Therapeutic Corp., Los Angeles, Calif.]), and serum from the patient obtained during his April 1993 admission before and during the administration of antibiotics.

RESULTS

Analysis of the patient's *C. fetus* isolate (strain 7307) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis demonstrated a band at 97 kDa representing a typical S-layer protein (1, 5). Consistent with this observation, strain 7307 was resistant to killing by normal human serum ($\log_{10}$ killing, 0.08). To assess the ability of serum from this chronically infected patient to provide opsonic functions, a series of opsonophagocytosis assays were performed. Both the patient's strain, 7307, and the control strain, *C. fetus* 23D, possessing an S-layer protein (S^+), were highly resistant to effective opsonization by normal human serum, whereas S^- strain 23B was completely opsonized regardless of the serum source (data not shown). Subsequent studies focused on the effects of incubating the S^+ strains with phagocytic cells in the presence of a variety of serum sources (Table 1). Under these conditions, HI normal human serum induced essentially no killing of the S^+ strains, as expected, and was used to standardize results for other serum sources studied in parallel assays. Without heat inactivation, there was little added killing of these strains (data not shown).

TABLE 1. Effect of serum source on *C. fetus* survival in opsonophagocytosis assay

Serum source	Mean ± SEM % survival of *C. fetus* cells at 60 min[a]	
	Strain 23D	Strain 7307
HI normal human serum[b]	100	100
HI immune rabbit serum[c]	16.5 ± 4.1	36.9 ± 15.8
Immune rabbit serum	11.2 ± 0.9	10.3 ± 1.6
HI patient serum[d]	77.0 ± 11.8	50.7 ± 8.3
HI i.v. Ig prepn 1	82.0 ± 12.7	68.6 ± 10.7
HI i.v. Ig prepn 2	90.9 ± 9.1	56.6 ± 11.9

[a] Inocula of 1,000 to 3,000 *C. fetus* cells in 10 μl of Hanks balanced salt solution were incubated for 60 min with 170 μl of whole blood from a healthy donor with no history of *C. fetus* infection. Aliquots were then cultured to determine viable cell counts. Survival results from two to four runs were normalized by comparison with simultaneous results obtained with HI normal human serum as the negative control.

[b] At 56°C for 30 min.

[c] Immune serum raised against 97-kDa *C. fetus* S-layer protein.

[d] Serum obtained from case person on day 4 of illness, prior to administration of antimicrobial agents.

A second serum sample (HI-P2) from the patient, obtained while he was receiving imipenem, showed more killing than the first serum sample from the patient but not at the level of immune serum (data not shown). Without heat inactivation, serum samples from the patient contained slightly more opsonic activity but still far less than did immune serum (data not shown).

To address the question of whether commercial i.v. Ig preparations contain antibodies which enhance killing of *C. fetus*, the above-described studies were repeated with addition of such preparations as the potential opsonins. Killing of *C. fetus* in the presence of i.v. Ig preparations was no greater than with the patient's serum alone (Table 1). The results obtained with untreated i.v. Ig and HI i.v. Ig were similar (data not shown).

DISCUSSION

The present case emphasizes many clinical features of *C. fetus* bacteremia. Fever is usually present, and gastrointestinal symptoms occur in less than 50% of patients (4, 6). Other systemic manifestations are myriad and include thrombophlebitis, cellulitis, cholangitis, osteomyelitis, endocarditis, and pneumonia. The typical host is a middle-aged to elderly man with some degree of immunosuppression, and mortality in this population approaches 25% (12). Antibiotic selection is guided by susceptibility of the individual strain, although the optimal duration of therapy is not well defined. Notably, progression of *C. fetus* osteomyelitis in a patient receiving erythromycin therapy has been previously reported (4).

This case has several unique features that contribute to our understanding of the pathogenesis of this disease. We believe that the patient's right clavicle was damaged by thoracic surgery and radiation in 1985 and was subsequently seeded during the postoperative episode of bacteremia. Erythromycin, a bacteriostatic agent, most likely cleared the systemic infection but did not clear a bony focus. *C. fetus* remained sequestered in the clavicle until many years later, when the patient developed hypogammaglobulinemia and received corticosteroids. At that point, his latent infection apparently reactivated, with resulting bacteremia. The absence of specific antibodies in this man and the intrinsic resistance of *C. fetus* to complement-mediated killing (2, 3), we believe, enabled the pathogen to

cause a persistent infection. While a bactericidal agent may have prevented the recurrence of *C. fetus* infection in this patient, it was unable to eradicate the infection after development of hypogammaglobulinemia.

The in vitro studies support these clinical observations. Because of the presence of S-layer proteins (2, 3), the patient's strain was serum resistant, facilitating the development of bacteremia (1). Because of the S-layer-induced complement resistance (3), specific antibodies are necessary to eradicate this extracellular infection by permitting killing by phagocytic cells. The opsonophagocytosis assay used provides a direct means for evaluating the role of specific antibodies in *C. fetus* clearance and for assessing the susceptibility of particular strains. Reflecting his underlying immunopathy, this patient did not produce functional antibodies either to his strain or to another S^+, strain despite being infected with *C. fetus* for several years. The absence of such antibodies explains why the infection could not be cured in the last year.

That commercial i.v. Ig preparations contained no more functional opsonic activity toward *C. fetus* than did pooled normal serum or serum from the infected patient suggests that i.v. Ig would not be beneficial in the treatment of hypogammaglobulinemic patients who have *C. fetus* bacteremia, in contrast to its apparent utility for *C. jejuni* bacteremia (8). One possible explanation for this discrepancy is that *C. jejuni* is a pathogen to which many adults have been exposed, and a specific antibody would therefore be expected in immunoglobulin preparations. In contrast, *C. fetus* infection is uncommon in humans, and specific antibodies are thus less likely to be present in random, pooled Ig preparations.

The decision to treat with lifelong antibiotic suppression was based on the in vitro failure of Ig to opsonize the patient's strain of *C. fetus* and the impression that during his multiple episodes of bacteremia, additional body sites had been seeded. Since a reversible cause for his immunosuppression is not present, the risk for *C. fetus* bacteremia and its consequences will probably be lifelong.

C. fetus causes a wide variety of clinical syndromes, and this case illustrates its propensity to affect immunocompromised hosts and its ability to cause osteomyelitis and bacteremia. The intrinsic serum resistance of the patient's *C. fetus* strain, the probable presence of a sequestered site, and his inability to form specific Igs account for the prolonged and relapsing nature of his infection. On the basis of our in vitro analysis, commercially obtained i.v. Ig preparations are not likely to be useful in the eradication of *C. fetus* infections.

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