Clinical Immunology
Vol. 97, No. 2, November, pp. 121–129, 2000
doi:10.1006/clim.2000.4932, available online at http://www.idealibrary.com on IDEAL®

Bacteremia and Skin/Bone Infections in Two Patients with X-Linked Agammaglobulinemia Caused by an Unusual Organism Related to *Flexispira*/*Helicobacter* Species

Brenda Cuccherini,* Kevin Chua,* Vee Gill,† Susan Weir,† Betty Wray,‡ Donn Stewart,§ David Nelson,§ Ivan Fuss,* and Warren Strober*

**Mucosal Immunity Section, Laboratory of Clinical Investigation, NIAID, †Microbiology Service, Department of Clinical Pathology, and §Immunophysiology Section, Metabolism Branch, NCI, National Institutes of Health, Bethesda, Maryland 20892; and ‡Medical College of Georgia, Augusta, Georgia 30912*

Two patients with Bruton's X-linked agammaglobulinemia are described with bacteremia and skin/bone infection due to an organism which by 16S rRNA gene sequence analysis was most closely related to *"Flexispira" rappini* (and thus designated a Flexispira-like organism, FLO) and more distantly related to the *Helicobacter* species. The organism required microaerobic conditions and, supplemental H_2 gas for growth and was reliably stained with acridine orange. In common with *Helicobacter cinaedi* infections, the focus of the FLO infection was in one case in the blood vessels or lymphatics of an extremity and in the other case in the skin and adjacent bone of an extremity. In both cases, prolonged IV antibiotic therapy was necessary to clear the infection. The susceptibility of XLA patients to FLO infection appears to be related to the fact that XLA is associated with severe B cell (humoral) immunodeficiency and thus these patients have difficulty with intravascular or intralymphatic infection. These findings elucidate the nature of FLO infections in humans and point the way to their detection and treatment.

Key Words: **agammaglobulinemia; bacteremia; Helicobacter; immunodeficiency.**

INTRODUCTION

In this paper we describe two patients with Bruton's X-linked agammaglobulinemia (XLA) who had bacteremia and several unique system manifestations due to infection with an unusual "*Flexispira*"-like organism (FLO). *Flexispira* species are closely related to the genus *Helicobacter*, and the bacteriologic and molecular characteristics of one of these organisms have been described previously (1). Here we describe a second case and provide a more in-depth description of the clinical and immunologic characteristics of the patients, relating the pathogenesis of the infection to the underlying immunodeficiency.

XLA is a primary immunodeficiency disorder characterized by either an absence or a very severe deficiency of B cells, consequently resulting in a lack of normal antibody production (2–4). This disorder is due to a mutation of a gene on the X-chromosome encoding a tyrosine kinase (Btk) specific to B cells and monocytes, which is necessary for the maturation of early B cells (pre-B cells) (5–8). As a result of this B cell disorder, the patients develop frequent bacterial infections of the upper and lower respiratory tracts that are primarily due to encapsulated bacteria such as *Haemophilus influenzae* and *Streptococcus pneumoniae*. Patients usually do not have an increased difficulty with viral infections except for those that enter via the gastrointestinal tract and disseminate hematogenously, such as poliovirus, some enteroviruses, and the hepatitis viruses (9). The primary treatment of XLA is administration of intravenous immunoglobulin with at least 400 mg/kg for 21 days (10, 11).

Although XLA and other B cell deficiencies are also associated with enteric infections due to *Giardia* and *Campylobacter* (9), there have been no reported cases of sepsis with the *Helicobacter* species. However, a review of the literature reveals that other immunodeficiencies have been associated with systemic infections with *Helicobacter* organisms, the majority occurring in individuals with HIV-induced immunodeficiency. The species isolated have included *Helicobacter westmeadii* (12), *Helicobacter* strain *Mainz* (13, 14), and most frequently, *Helicobacter cinaedi* (15–23). Two reports of bacteremia caused by "*Flexispira rappini*" have occurred, one in a normal 9-year-old female with pneumonia (24) and the other in a 65-year-old male hemodialysis patient with HIV infection (25). The two FLO strains isolated from our XLA patients were found to be closely related to each other and belong to the same species, and although they are closely related to *F. rappini*, they are thought to represent a different species altogether (1).

1521-6616/00 $35.00

Case Reports

Case 1. The first patient (G.M., reported previously in Ref. 1) was a 36-year-old black male with XLA and a history of chronic sinusitis, chronic hepatitis B and C, and chronic prostatitis. A brief history of his leg symptoms and FLO infection is as follows: G.M. experienced transient episodes of leg swelling from early childhood that continued up to his most recent admissions for persistent leg swelling and inflammation. In 1994, a diagnosis of cellulitis was presumed after the appearance of increased right ankle and calf edema, leading to antibiotic treatment on several occasions. In 1996 (age 34), he was seen at NIH where a right leg lesion characterized by increased pigmentation and "woody" or ligneous swelling of both the calf and the thigh (not associated with increased warmth) was noted (Figs. 1A and 1B). Blood cultures at this point were positive for a *Helicobacter*-like organism (not *Helicobacter pylori*) that was eventually identified as FLO. Subsequently, the patient was treated with multiple courses of antibiotics for episodes of sepsis with this organism, including courses of ampicillin/sulbactam, trimethoprim/sulfamethoxazole, ciprofloxacin, clarithromycin, doxycycline, metronidazole, minocycline, and rifampin. Nevertheless, blood cultures remained positive for FLO, and there was only limited improvement in the leg edema. The multiresistance of this isolate to a variety of antibiotics narrowed therapeutic options to imipenem, gentamicin, minocycline, and metronidazole. On subsequent testing of later isolates, the organism remained susceptible to imipenem, gentamicin, and minocycline but had become resistant to metronidazole (>32 μg/ml). The patient had received all of these antimicrobial agents. In early 1997, he was started on a 5-month course of iv gentamicin and imipenem. On this regimen, blood cultures became and remained negative during an 18-month follow-up period. In addition, systemic symptoms subsided, and leg edema decreased substantially, although the minimal swelling and a "ligneous" appearance persisted. Of interest, G.M. has a brother with XLA who had a chronic skin infection of the left lower leg or ankle, eventually leading to ulceration. However, a specific organism was not grown from either the leg lesion or the blood.

Case 2. The second patient (G.Q.) is a 21-year-old Hispanic male who developed recurrent pneumonia, sinusitis, and otitis media—as well as diarrhea and failure to thrive at the age of 6 months. At that time, examination revealed lack of tonsillar tissue and low IgA, IgG, and IgM levels, which led to a diagnosis of XLA and the institution of im immunoglobulin and iv therapy. At age 13, the patient noted the onset of left knee swelling followed later by the onset of right ankle swelling, erythema, and pain. This led to an open synovial biopsy that revealed nonspecific proliferative synovitis that was negative on culture. The ankle swelling continued, gradually progressing to involve the adjacent leg area and ultimately leading to the formation of nonhealing skin ulcers. At age 17, a diagnosis of pyoderma gangrenosum (Fig. 1D) was made, which led to treatment with oral steroids.

At age 18, the patient had the of onset fevers that led to iv treatment with gentamicin, metronidazole, and vancomycin. As a result, the fever resolved and the above-mentioned right lower extremity (RLE) lesions healed, but returned upon cessation of therapy and were accompanied by an evanescent erythematous, macular skin rash distributed in a random pattern over the extremities (Fig. 1C).

At age 21, the patient noted left knee pain and swelling and was placed on iv vancomycin. Biopsies of the bone and synovium disclosed both acute and chronic synovitis, and acute and chronic osteomyelitis of the femur, but were culture-negative. Esophogastroduodenoscopy at this point was positive for *H. pylori* and the patient was treated with clarithromycin and omeprazole which led to clearance of the *H. pylori*. Subsequently, ultrasound studies revealed poor venous flow into the liver and splenomegaly, leading ultimately to splenectomy; pathologic examination of the latter revealed marked lymphoid depletion, absence of B cells, and extramedullary hematopoiesis.

At age 21, the patient was admitted to the NIH (NIAID) for further evaluation. Here, blood cultures were positive on several occasions for a Gram-negative curved rod thought to be a *Helicobacter*-like organism and subsequently identified as FLO; however, antibiotic susceptibility testing was unsuccessful because the organism was unusually fastidious. Biopsy of the RLE area of ulceration revealed inflammation consistent with the healing phase of an ulcer and pyoderma granulosum; biopsy of the macular rash revealed nonspecific inflammation, possibly due to urticarial reaction or drug eruption. An MRI and a bone scan of the lower extremities suggested the presence of osteomyelitis of the distal left femur, proximal left tibia, and the right calcaneus. These findings, along with the positive blood cultures, led to surgical debridement of the femur. Specimens obtained during the procedure were positive for the same Gram-negative curved rod grown from the blood.

Treatment with intravenous imipenem and gentamicin was begun and led, initially, to resolution of the fevers and the macula rash and later to gradual improvement in the RLE ulcers. However, the gentamicin was discontinued because of hearing loss and was replaced by intravenous meropenem. After approximately 9 months of iv antibodies, there was substantial improvement in the RLE ulcers and therapy was discontinued.

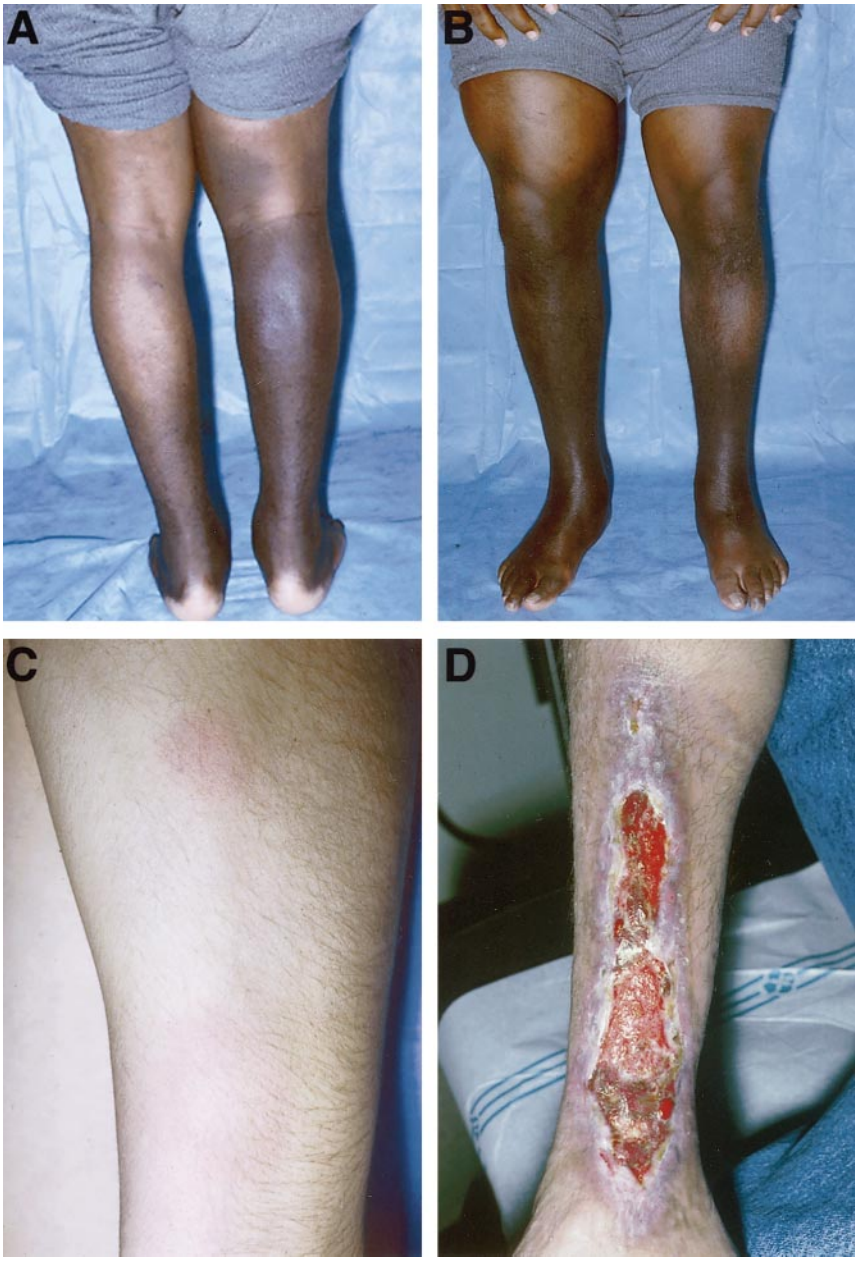

FIG. 1. (A, B): Back and front views of lower limbs of patient G.M. showing hyperpigmentation and swelling of the right lower limb (calf and thigh). (C) Evanescent erythematous rash on the left lower leg of patient G.Q. (D) Chronic ulcerative lesion (pyoderma-like lesion) on the right lower leg of patient G.Q.

MATERIALS AND METHODS

Subjects and Specimen Preparation

Peripheral blood mononuclear cells (PBMCs) were obtained by lymphapheresis from normal adult volunteers and patients and then further purified for studies of $CD4^+$ T cell proliferation and cytokine production as previously described (26).

Proliferation and Cytokine Production Assays

Cell cultures were performed to measure T cell proliferation and cytokine production as previously described (26). To measure $CD4^+$ T cell proliferation, $CD4^+$ cells were stimulated with anti-CD28 plus either anti-CD2 or anti-CD3 in 96-well flat-bottom plates, harvested, and counted in a liquid scintillation counter

as previously described (26). To measure cytokine production, PBMCs and PB $CD4^+$ T cells were cultured in 24-well plates and stimulated with anti-CD28, and either anti-CD2 or anti-CD3, as previously described (26). After 48 h, supernatants were removed and assayed for IL-2, IL-4, IL-5, IL-10, and IFN-γ using commercially available ELISA kits, following methods outlined by the manufacturer (R&D Systems, Minneapolis, MN). For analysis of IL-12, cell populations were prestimulated with IFN-γ for 18 h and subsequently with SAC for an additional 24 h. Supernatants were then removed and assayed by a commercially available ELISA kit (R&D Systems).

Western Blot Assay

Isolated PBMCs were lysed at a concentration of 4×10^7 cells in 100 μl of PBS (Life Technologies, Gaithersburg, MD) containing 1% Triton X-100 (Research Products International, Mount Prospect, IL), 1 mM PMSF (Sigma, St. Louis, MO), and 10 μg/ml aprotinin (Sigma) on ice for 15 min followed by centrifugation at maximum speed for 1 min in a microcentrifuge. The supernatant was isolated and added to an equal volume of SDS sample buffer containing 2% 2-mercaptoethanol and heated at 100°C for 5 min. A total of 15 μl of sample was loaded onto a 1-mm 4–20% SDS polyacrylamide gel (Novel Experimental Technology, San Diego, CA) and transferred to an Immobilon-P PVDF membrane (Millipore, Bedford, MA) by semidry transfer at 15 V for 2 h in Novex transfer buffer (Novel Experimental Technology) containing 10% methanol. The blot was washed three times in T-TBS (0.1 M Tris, pH 7.4, 0.3 M NaCl, 0.1% Tween 20) for 5 min and blocked overnight in T-TBS containing 0.2% casein. The membrane was then probed with monoclonal anti-BTK (8E5-A10) (27) at a concentration of 5 μg/ml for 1 h, washed three times in T-TBS, and subsequently incubated with a goat anti-mouse IgG horseradish peroxidase conjugate (Life Technologies) at a 1:5000 dilution for 1 h. After being washed further, the blot was developed for 1 min with 5 ml of SuperSignal (Pierce, Rockford, IL) substrate for 1 min, drained, and then exposed to Kodak BioMax MR film for 3 min. After exposure, the blot was washed with T-TBS and reprobed with an anti-WASP monoclonal (3F3-A5) (28) at a concentration of 2 μg/ml as above, to insure that samples were equally loaded on the blot. The blot was not stripped before reprobing.

Organism Identification

Biochemical, electronmicroscopic, and molecular characterization and identification of the organism in case 1 (FLO-1) have been previously described (1). Identification of organism 2 (FLO-2) was performed using the same methodology. Results of the biochemical and microscopic characteristics are shown in Table 1, which also compares these isolates to other Helicobacter and to *F. rappini*. Gene sequencing of the 16S rRNA of FLO-2 was done at the NIH by the previously described method, while whole cell DNA-DNA hybridization studies between the FLO-1 and FLO-2 and between each FLO and *F. rappini* were done at the CDC.

Antibiotic Susceptibility Testing

The fastidious nature of the organism precluded the use of traditional disk or microdilution methods. Instead, *in vitro* antibiotic susceptibility testing was performed with the use of E-test strips (AB Biodisk, Piscataway, NJ) placed on heavily inoculated sheep blood agar and incubated in a microaerophilic atmosphere with H_2. The E-test strips were generally read after 2 to 3 days of incubation. Along with the patient's organism, control organisms for which the MICs were known were tested with the same conditions and medium to validate the expected activities of the antibiotics. Although there are no National Committee for Clinical Laboratory Standards recommended breakpoints for this organism, susceptibility or resistance for each antibiotic was estimated based on MIC breakpoints used for other organisms. The following agents were tested: amoxicillin–clavulanate, ampicillin, azithromycin, ceftriaxone, ciprofloxacin, clindamycin, chloramphenicol, doxycycline, gentamicin, imipenem, minocycline, and metronidazole. β-Lactamase production was tested for by using DrySlide nitrocefin (Difco, Detroit, MI). Susceptibilities were determined with the first blood isolate recovered from patient G.M. at NIH in August 1996.

RESULTS

Immunologic Characterization of Patients G.M. and G.Q.

The two patients described here were assigned the diagnosis of XLA on the basis of strong evidence for the presence of a defect in the BTK gene. Patient G.M. had a male sibling with a similar B cell defect and was shown on Western blot of postnuclear supernatant using a mAb specific for BTK to produce abnormally low amounts of a BTK molecule (Fig. 2). Subsequent sequencing of this patient's BTK cDNA showed a 33-base-pair deletion (cDNA bases 2008–2040) of exon 18 at the exon 18–19 junction. This in-frame deletion results in a BTK protein missing amino acids 626–636, thereby resulting in a protein with impaired function and/or stability. Sequence analysis of a genomic PCR

TABLE 1

Microbial Characterization of "*Flexispira*"-like Organism (FLO)

Test	FLO-1	FLO-2	"*F. rappini*"	*H. cinaedi*	*H. pylori*
Catalase	+	+	+ or −	+	+
Oxidase	+	+	+	+	+
Nitrate	−	−	−	+	+ or −
Reduction					
Urease	+	+	+	−	+
Alkaline phosphatase	+	+	−	−	+
Hippurate hydrolysis	−	−	−	−	−
Growth at					
25°C	−	−	−	−	−
35°C	+	+	+	+	+
42°C	−	−	+	−/weak	+
Nalidixic acid	Resistant	Resistant	Resistant	Sensitive	Resistant
Cephalothin	Resistant	Resistant	Resistant	Intermediate	Sensitive
Periplasmic fibers	+	+	+	−	−
No. of Flagella	8–12	8–12	10–20	1–2	4–8
Distribution	Bipolar	Bipolar	Bipolar	Bipolar	Bipolar

product encompassing exon 18 and part of introns 17 and 18 revealed that exon 18 was intact, but there was a G to T mutation in the conserved first position of intron 18. This results in the use of a cryptic splice site within exon 18 at cDNA position 2007, causing the deletion of the remainder of exon 18. Patient G.Q. had no family history of similar diseases but was shown on Western blot analysis of postnuclear supernatant to express little or no BTK (Fig. 2). The diagnosis of XLA in both patients was corroborated by the fact that using flow cytometric analysis they had virtually no circulating B cells.

Flow cytometric studies of T cell phenotypes in the two patients disclosed that while both patients had normal numbers of $CD3^+$ T cells, one of the two patients, G.M., had a reversed CD4/CD8 T cell ratio (0.41) and elevated numbers of $CD8^+$ T cells which expressed a marker of previous activation (HLA-DR) or differentiation (CD57). Because this patient also had chronic hepatitis B and C infection, these T cell findings probably represent a response to intracellular viral infection rather than a response to the FLO infection.

Studies of T cell function (see Materials and Methods) revealed that both patients' T cells exhibited normal proliferative responses following polyclonal stimulation with anti-CD3 or anti-CD2 plus anti-CD28, in comparison with two simultaneously studied normal cell populations. In addition, studies of patient T cell cytokine production (IFN-γ, IL-4, IL-5, and IL-10) also appeared to be normal or even somewhat increased (with respect to IL-4/IL-5 production), again in comparison with two simultaneously studied normal cell populations. Finally, stimulation of patient PBMCs with *S. aureus* Cowan 1 (SAC) and IFN-γ revealed normal production of IL-12, once again in comparison with PBMC from two simultaneously studied normal donors. The normal T cell function disclosed by these studies strongly suggests that abnormalities of T cell function do not account for the occurrence of FLO infection in these patients.

Microbiologic Characterization of Helicobacter-like Species from Patients G.M. and G.Q.

Both patients were bacteremic with curved Gram-negative rods that were slow growing and required enriched media and a microaerobic atmosphere that in addition to reduced oxygen levels also contained some H_2 gas. Fortunately, both organisms grew in aerobic blood culture bottles (BTA, Organon-Teknika), with positive detection occurring between 2 and 8 days of incubation. Organisms were not reliably detected by the automated detection system due to their slow growth curves and were not reliably seen by Gram stain. Staining of blood cultures using acridine orange stain yielded the fastest and most reliable detection of positive cultures. Subcultures to blood or chocolate

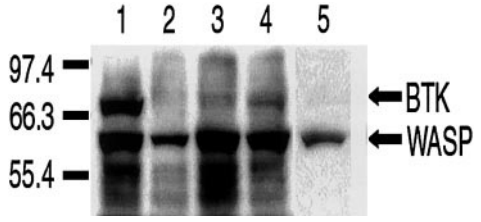

FIG. 2. Western blots of postnuclear supernatants of patients with XLA and a control patient. Lane 1: normal control; lanes 2 and 3 (MW and HK): patients with known XLA; lane 4: patient G.M.; lane 5: (patient G.Q.) BTK, Bruton's tyrosine kinase; WASP, Wiskott–Aldrich syndrome protein (control protein).

agar plates were successful only when incubated at 35–37°C under microaerobic conditions—as are used for *Campylobacter* or *Helicobacter* isolation—as long as H_2 was included in the atmosphere. The organisms from both patients were similar to each other morphologically and by several biochemical characteristics, shown in Table 1, which for comparison also shows the characteristics for *F. rappini*, *H. cinaedi*, and *H. pylori*.

Molecular-based identification using 16S rRNA gene sequence analyses showed that each of the FLO isolates was most closely related to an organism currently designated *F. rappini*, which may be a generically misnamed member of the genus *Helicobacter*. However, the two patient FLO isolates were more closely related to each other than to other *Helicobacter* sp. and *F. rappini*. By DNA-DNA hybridization studies done at the CDC, the two patient strains showed 81% relatedness, a level sufficient to regard them as the same species. In contrast, both strains had <70% homology with *F. rappini*, indicating that FLO represents a new taxon distinct from *F. rappini*. At present, it is unclear as to whether FLO-1 and FLO-2 will be included as members of the genus *Helicobacter*, thus we have designated them *Flexispira*-like organisms; however, as outlined in our previous publication relating to FLO, it should be emphasized here that FLO organisms are not in fact *Flexispira sp*. Regardless of whether FLO organisms are present in the normal intestinal flora, it seems likely that these organisms are widely disseminated in the environment and thus infection depend more on patient susceptibility than the abnormal exposure. This is underscored by the fact that there was no history of unusual exposure to FLO organisms in either patient.

DISCUSSION

This is the first clinical report of systemic infection caused by FLO, a new and uncommon bacterial species, in two patients with severe B cell immunodeficiency. Molecular studies with FLO-1 and FLO-2 reveal them to be more closely related to *F. rappini* than to named *Helicobacter* species such as *H. cinaedi* or *H. pylori*. This relatedness is further supported by the distinctive morphologic features that FLO shares with *F. rappini*, which include periplasmic fibers along with four or more flagella on each end of the organism ("bipolar"). Although there are at least four other species of *Helicobacter* with demonstrated periplasmic fibers and bipolar flagella, they are less closely related to FLO by 16S rRNA sequence homology and have been isolated from nonhuman mammals only.

For a comparison of clinical features of systemic infections caused by other closely related organisms, we reviewed infections caused predominantly by *H. cinaedi* and two cases caused by *F. rappini*. Of 7 patients with *H. cinaedi* bacteremia reported by Burman *et al.* (20), 4 presented with multiple types of skin lesions (erythema nodosum, large erythematous plaques, and cellulitis), while Kiehlbauch *et al.* (21) described fever-associated macular rashes as well as "atypical" cellulitis marked by brown- or copper-colored skin without warmth in 9 of 23 cases. Of note, the majority of these cases occurred in immunocompromised HIV-positive patients. An additional case reported by Tee *et al.* (22) described an HIV-positive patient with *H. cinaedi* bacteremia who initially manifested lower limb culture-negative cellulitis, progressing to chronic lymphedema.

Only four cases of *F. rappini* infection have been reported, two in patients with gastroenteritis and chronic diarrhea in which *F. rappini* was cultured from the stool (29) and two in patients with bacteremia, one in a child with pneumonia of unknown etiology (24) and one in an HIV patient on hemodialysis (25), both of whom had *F. rappini* cultured from the blood. In the case report of the child, the primary clinical findings were fever, malaise, and cough associated with a lobar pneumonia; there were no skin or musculoskeletal manifestations associated with the infection. The HIV patient presented with chills and fever and was treated for *F. rappini* sepsis, but had recurrent sepsis 21 days later. He had a prior history of repetitive infected skin lesions of the lower limbs, diagnosed as chronic erysipelas, but more recently had suffered cellulitis of the forearm secondary to a cat scratch. The portal of entry of *F. rappini* is thought to be the patient's GI tract because the organism has previously been found in the GI tract of laboratory mice as well as in humans (29–32).

Patient G.M.'s dermatologic findings were similar to those of *H. cinaedi* patients who developed a "cold" cellulitis that progressed to a "woody"-appearing skin lesion suggestive of lymphatic obstruction. In contrast, patient G.Q.'s dermatologic findings were more unique. In his case, fevers were associated with evanescent macular rashes, but these occurred in association with chronic pyoderma granulosum-like lesions, a manifestation that has not been seen previously. A prominent feature of patient G.Q.'s clinical course was joint and bone infection. This has been previously reported in cases of *Helicobacter* and *Campylobactor* bacteremias, albeit infrequently. In the seven patients with *H. cinaedi* bacteremia reported by Burman *et al.* (20), two had monoarticular, large-joint arthritis. Similarly, of the two patients reported by Vandamme *et al.* with *H. cinaedi* bacteremia, one had monoarticular arthritis and the other a septic hip joint (17). Van der Ven *et al.* (23) reported a patient with soft tissue pain in the lower extremity without cellulitis in scintigraphy and an MRI that showed increased perfusion and the presence of soft tissue vascular structures. Furthermore, Kerstens *et al.* (33) reported a patient with XLA who

had *Campylobacter jejuni* bacteremia and a several-year history of recurrent fevers, who ultimately developed erysipelas-like lesions in the lower legs associated with scintigraphic evidence of osteomyelitis. Finally, Simon and Markusse (34) reported the occurrence of septic arthritis related to *C. jejuni* bacteremia in a patient with rheumatoid arthritis and XLA.

The association of FLO infection with XLA in particular, or infection with *Helicobacter*-like organisms with immunodeficiency in general has not been specifically noted previously, except for the observation that *H. cinaedi* infections occur most frequently in HIV patients. The susceptibility of XLA patients to the FLO infection of the extremities described here may be due to their extreme B cell (humoral) immune defect coupled with their completely normal T cell (cellular) function. Thus, as noted in both patients G.M. and G.Q., patients with XLA have virtually no B cells in the blood or tissues but have a normal number of T cells and monocytes which exhibit normal cytokine secretion. Patients with XLA contrast with patients with common variable immunodeficiency (CVI) whose B cell function is decreased but is not absent and who frequently have somewhat deficient T cell function. The immunodeficiency profile of XLA is probably responsible for the fact that XLA patients exhibit an increased incidence of chronic enteric infection, particularly infection due to Campylobacter and enteroviruses (9), i.e., superficial infections that require specific antibody responses for resolution. In addition, XLA patients are subject to bacteremias, particularly with organisms that colonize the gastrointestinal tract (33–41). Such is not the case in CVI although it occurs in AIDS (12–16, 18, 21–23, 25, 42, 43), perhaps because in the latter case a B cell deficiency occurs secondary to a severe T cell deficiency that is as profound as in XLA. That the origin of the FLO infection in the XLA patients studied here was the gastrointestinal tract is supported by the fact that, as mentioned, *Helicobacter* or *Helicobacter*-like organisms including *F. rappini* have been found to colonize the gastrointestinal tract of mice.

Whether the FLO organisms will be identified as a component of the normal intestinal flora of humans remains to be seen. It seems likely that the B cell deficiency of XLA patients also explains the somewhat unusual nature of the infection of the extremities with FLO and perhaps *H. cinaedi* as well. Thus, it appears that blood vessels and lymphocytes provide not only a conduit of spread, but also a focus of infection within these vascular/lymphatic structures. This possibility was evident in G.M., who exhibited cold cellulitis and woody lymphedema of the involved leg. In addition, the infection of the skin and bone seen in G.Q. could have arisen from prior intravascular infection. Of interest, in both G.M. and G.Q., FLO infection was limited to the lower extremities and was conspicuously absent in their internal organs. Although a peripheral or skin pattern of infection may be correlated with a lower growth temperature optimum of the infectious agent, our *in vitro* laboratory studies of FLO showed equivalent growth at 37 and 35°C.

The two cases presented here illustrate the need to consider infection with *Helicobacter*/*Flexispira* species in immunodeficient patients with unexplained fever—particularly when the latter is associated with dermatologic findings or musculoskeletal infection. Moreover, the index of suspicion specifically for FLO should be heightened if these manifestations occur in a patient with XLA. In suspected cases, blood cultures should be obtained using an aerobic blood culture bottle (preferably a BacT Alert system) incubated for at least 10 days and stained by acridine orange stain prior to discarding. If curved or spiral organisms are observed, they should be subcultured using enriched blood agar under microaerobic conditions that contain H_2, and plates should be incubated at 37°C for at least 7 days. Selective media for isolation of *Campylobacter* usually contain antibiotics and should not be used. Empiric antibiotic coverage should be initiated promptly since *in vitro* antibiotic susceptibility testing may be difficult to obtain. On the basis of the recurrent bacteremias observed in our two patients, we would recommend treatment with iv imipenem or meropenem in combination with an aminoglycoside such as gentamicin. Duration of therapy may need to be longer than standard and should be guided by studies of the organisms' sensitivities *in vitro*, the patient's response (decrease in fever or improvement of skin/bone lesions or cellulitis), and the maintenance of negative blood cultures. Both of the cases reported here were treated for up to 5 months.

In conclusion, FLO and other *Helicobacter* sp. must be added to the spectrum of unusual infections occurring in some B cell immunodeficiencies such as XLA. In these, the immunodeficiencies and the lack of organism-specific antibody may vitiate containment of the organism to gastrointestinal mucosal surfaces, leading to bacteremia and dissemination. Subsequent localization of the organism to vascular/lymphatic or musculoskeletal sites may be associated with overlying skin discoloration or swelling, with cellulitis or, rarely, with joint infections. For unknown reasons, these manifestations are common for systemic infections with this group of organisms and should help raise the index of suspicion for a *Helicobacter*-related infection and more specifically for an infection with FLO in the febrile XLA patient.

REFERENCES

1. Weir, S., Cuccherini, B., Whitney, A. M., Ray, M. L., MacGregor, J. P., Steigerwalt, A., Daneshar, M. I., Weyant, R., Wray, B., Steele, J., Strober, W., and Gill, V. J., Recurrent bacteremia

caused by a "Helicobacter"-like organism in a patient with X-linked (Bruton's) agammaglobulinemia. *J. Clin. Microbiol.* **37,** 2439–2445, 1999.

2. Bruton, O. C., Agammaglobulinemia. *Pediatrics* **9,** 722–728, 1952.
3. Conley, M. E., X-linked immunodeficiencies. *Curr. Opin. Genet. Dev.* **4,** 401–406, 1994.
4. Conley, M. E., Parolini, O., Rohrer, J., and Campana, D., X-linked agammaglobulinemia: New approaches to old questions based on the identification of the defective gene. *Immunol. Rev.* **138,** 5–21, 1994.
5. Rosen, F. S., Cooper, M. D., and Wedgwood, R. J. P., The primary immunodeficiencies (1 and 2). *N. Engl. J. Med.* (7) **311,** 235–242; (8) **311,** 300–310, 1984.
6. Tsukada, S., Saffran, D. C., Rawlings, D. J., Parolini, O., Allen, R. C., Klisak, I., Sparks, R. S., Kubagawa, H., Mohandas, T., Quan, S., Belmont, J. W., Cooper, M. D., Conley, M. E., and Witte, O. N., Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* **72,** 279–290, 1993.
7. Vetrie, D., Vorechovsky, I., Sideras, P., Holland, J., Davies, A., Flinter, F., Hammarstrom, L., Kinnon, C., Levinshky, R., Bobrow, M., Smith, C. I. E., and Bentley, D. R., The gene involved in X-linked agammaglobulinemia is a member of the src family of protein-tyrosine kinases. *Nature* **361,** 226–233, 1993.
8. Rohrer, J., Parolini, O., Belmont, J. W., and Conley, M. E., The genomic structure of human BTK, the defective gene in X-linked agammaglobulinemia. *Immunogenetics* **40,** 319–324, 1994.
9. Lederman, H. M., and Winkelstein, J. A., X-linked agammaglobulinemia: An analysis of 96 patients. *Medicine* **64,** 145–156, 1985.
10. Minegishi, Y., Rohrer, J., and Conley, M. E., Recent progress in the diagnosis and treatment of patients with defects in early B-cell development. *Curr. Opin. Pediatr.* **11,** 528–532, 1999.
11. Nowak-Wegryzn, A., and Lederman, H. M., Supply, use, and abuse of intravenous immunoglobulin. *Curr. Opin. Pediatr.* **11,** 533–539, 1999.
12. Trivett-Moore, N. L., Rawlinson, W. D., Yuen, M., and Gilbert, G. L., *Helicobacter westmeadii* sp. Nov., a new species isolated from blood cultures of two AIDS patients. *J. Clin. Microbiol.* **35,** 1144–1150, 1997.
13. Fleish, F., Burnens, A., Weber, R., and Zbinden, R., Helicobacter species strain Mainz isolated from cultures of blood from two patients with AIDS. *Clin. Infect. Dis.* **26,** 526–27, 1998.
14. Husmann, M., Gries, C., Jehnichen, P., Woelfel, T., Gerken, G., Ludwig, W., and Bhakdi, S., Helicobacter sp. Strain Mainz isolated from an AIDS patient with septic arthritis: Case report and nonradioactive analysis of 16S rNA sequence. *J. Clin. Microbiol.* **32,** 3037–3039, 1994.
15. Pasternak, J., Bolivar, R., Hopfer, R. L., Fainstein, V., Mills, K., Rios, A., and Bodey, G. P., Bacteremia caused by campylobacter-like organisms in two male homosexuals. *Ann. Inst. Med.* **101,** 339–341, 1984.
16. Ng., V. L., Hadley, W. K., Fennell, C. L., Flores, B. M., and Stamm, W. E., Successive bacteremia with "*Campylobacter cinaedi*" and "*Campylobacter fennelliae*" in a bisexual male. *J. Clin. Microbiol.* **25,** 2008–2009, 1987.
17. Vandamme, P., Falsen, E., Bot, B., Kersten, K., and De Ley, J., Identification of *Campylobacter cinaedi* isolated from blood and feces of children and adult females. *J. Clin. Microbiol.* **28,** 1016–1020, 1990.
18. Sacks, L. V., Labriola, A. M., Gill, V. J., and Gordin, F. M., Use of ciprofloxacin for successful eradication of bacteremia due to *Campylobacter cinaedi* in a human immunodeficiency virus-infected person. *Rev. Infect. Dis.* **13,** 1066–1068, 1991.
19. Orlicek, S. L., Welch, D. F., and Kuhls, T. L., Septicemia and meningitis caused by *Helicobacter cinaedi* in a neonate. *J. Clin. Microbiol.* **31,** 569–571, 1993.
20. Burman, W. J., Cohn, D., Reves, R. R., and Wilson, M. L., Multifocal cellulitis and monoarticular arthritis as manifestations of *Helicobacter cinaedi* bacteremia. *Clin. Infect. Dis.* **20,** 564–570, 1994.
21. Kiehlbauch, J. A., Tauxe, R. V., Baker, C. N., and Wachsmuth, I. K., *Helicobacter cinaedi*-associated bacteremia and cellulitis in immunocompromised patients. *Ann. Intern. Med.* **121,** 90–93, 1994.
22. Tee, W., Street, A. C., Spelman, D., Munckhof, W., and Mijch, A., *Helicobacter cinaedi* bacteremia: Varied clinical manifestations in three homosexual males. *Scand. J. Infect. Dis.* **28,** 199–203, 1996.
23. Van der Ven, A. J. A., Kullberg, B. J., Vandamme, P., and Meiw, J. F. G., *Helicobacter cinaedi* bacteremia associated with localized pain but not cellulitis. *Clin. Infect. Dis.* **22,** 710–712, 1996.
24. Tee, W., Leder, K., Karroun, E., and Dyall-Smith, M., "*Helicobacter rappini*" bacteremia in a child with pneumonia. *Clin. Microbiol.* **36,** 1679–1682, 1998.
25. Sorlin, P., Vandamme, P., Nortier, J., Hoste, B., Rossi, C., Paulof, S., and Struelens, M. J., Recurrent "*Helicobacter rappini*" bacteremia in an adult patient undergoing hemodialysis: Case report. *J. Clin. Microbiol.* **37,** 1319–1323, 1999.
26. Fuss, I. J., Neurath, M., Boirivant, M., Klein, J. S., de la Motte, C., Strong, S. A., Fiocchi, C., and Strober, W., Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *J. Immunol.* **157,** 1261–1270, 1996.
27. Stewart, D., Kurman, C., and Nelson, D., Production of monoclonal antibodies to Bruton's tyrosine kinase (BTK). *Hybridoma* **14**(3), 243–246, 1995.
28. Stewart, D., Treiber-Held, S., Kurman, C., Fachetti, F., Notarangelo, L., and Nelson, D., Studies of the expression of the Wiskott–Aldrich syndrome protein. *J. Clin. Invest.* **97**(11), 2627–2634, 1996.
29. Romero, S., Archer, J. R., Hamacher, M. E., Bologna, S. M., and Schell, R. F., Case report of an unclassified microaerophilic bacterium associated with gastroenteritis. *J. Clin. Microbiol.* **26,** 142–143, 1988.
30. Schauer, D. B., Ghori, N., and Falkow, S., Isolation and characterization of "*Helicobacter rappini*" from laboratory mice. *J. Clin. Microbiol.* **31,** 2709–2714, 1993.
31. Riley, L. K., Franklin, C. L., Hook, R. R., and Besch-Williford, C., Identification of murine Helicobacters by PCR and restriction enzyme analyses. *J. Clin. Microbiol.* **34,** 942–946, 1996.
32. Zenner, L., Pathology, diagnosis, and epidemiology of the rodent Helicobacter infection. *Comp. Immunol. Microbiol. Infect. Dis.* **22,** 41–61, 1999.
33. Kerstens, P. J. S. M., Endtz, H. P., Meis, J. F. G. M., Oyen, W. J. O., Koopman, R. J. L., van den Broek, P. J., and Van der Meer, J. W. M., Erysipelas-like lesions associated with *Campylobacter jejuni* septicemia in patients with hypogammaglobulinemia. *Eur. J. Clin Microbiol. Infect. Dis.* **11,** 842–847, 1992.
34. Simon, C. H., and Markusse, H. M., *Campylobacter jejuni* arthritis in secondary amyloidosis. *Clin. Rheum.* **14,** 214–216, 1995.
35. Melamed, I., Bujanover, Y., Igra, Y. S., Schwartz, D., Zakuth, V., and Spirer, Z., Campylobacter enteritis in normal and immunodeficient patients. *Am. J. Dis. Child.* **137,** 752–753, 1983.

36. Neuzil, K. M., Wang, E., Haas, D. W., and Blaser, M. J., Persistence of campylobacter features bacteremia associated with absence of oposinizing antibodies. *J. Clin. Microbiol.* **32,** 1718–1720, 1994.
37. LeRisbe, C., Bosseray, A., Lecerq, P., Pegourie, B., Megraud, F., and Croize, J., Relapsing *Campylobacter coli* bacteremia in a hypogammaglobulinemia patient. *Presse. Med.* **27,** 1103–1104, 1998.
38. Chusid, M. J., Wortmann, D. W., and Donne, W. M., *Campylobacter upsaliensis* sepsis in a boy with acquired hypogammaglobulinemia. *Diagn. Microbiol. Infect. Dis.* **13,** 367–369, 1990.
39. Chusid, M. J., Coleman, C. M., and Dunne, W. M., Chronic asymptomatic campylobacter bacteremia in a boy with X-linked hypogammaglobulinemia. *Ped. Infect. Dis. J.* **6,** 943–944, 1987.
40. Asmar, B. I., Andregen, J., and Brown, W. J., Ureaplasma urealyticum arthritis and bacteremia in agammaglobulinemia. *Ped. Infect. Dis. J.* **17,** 73–76, 1998.
41. Meyer, R. D., and Clough, W., Extragenital *Mycoplasma hominis* infection in adults: Emphasis on immunosuppression. *Clin. Infect. Dis.* **17,** 234–249, 1993.
42. Perlman, D. M., Ampel, N. M., Schifman, R. B., Cohn, D. L., Patton, C. M., Aguirre, M. L., Wang, W. L., and Blaser, M., Persistent *Campylobacter jejuni* infections in patient infected with human immunodeficiency virus (HIV). *Ann. Intern. Med.* **108,** 540–556, 1988.
43. Johnson, R. J., Nolan, C., Wang, S. P., Shelton, W. R., and Blaser, M. J., Persistent *Campylobacter jejuni* infection in an immunocompromised patient. *Ann. Intern. Med.* **100,** 832–834, 1984.

Received April 27, 2000; accepted August 9, 2000