BRIEF REPORTS

Disseminated *Flavimonas oryzihabitans* Infection in a Diabetic Patient Who Presented with Suspected Multiple Splenic Abscesses

Flavimonas oryzihabitans is an aerobic, mobile, yellow-pigmented, oxidase-negative, gram-negative rod that was formerly known as CDC (Centers for Disease Control) group Ve-2, *Chromobacterium typhiflavum,* and *Pseudomonas oryzihabitans* [1]. The most common infections caused by these organisms are prosthetic valve endocarditis, peritonitis (in patients undergoing continuous ambulatory peritoneal dialysis), and bacteremia secondary to indwelling catheters and neurosurgical shunt infections [2]. Community-acquired infections caused by *F. oryzihabitans* are rare and involve soft tissue [3]. We report a case of *F. oryzihabitans* sepsis in a diabetic adult who presented with suspected multiple splenic abscesses.

A 53-year-old man presented to another hospital in the city with a 4-week history of intermittent fever (temperature to 40°C), malaise, and a 10-kg weight loss. Diabetes and upper respiratory tract infection were diagnosed, and oral antidiabetics and ampicillin (2 g/d for 10 days) were prescribed; however, the patient's condition did not improve and he was admitted to our hospital.

Physical examination revealed a middle-aged man who complained of weakness and had an oral temperature of 37.5°C. The spleen was palpable 2 cm below the left costal margin with a span of 12 cm. The WBC count was $17{,}500 \times 10^9$/L, and the fasting blood glucose level was 251 mg/dL. An abdominal ultrasonogram revealed round, well-demarcated multiple cystic lesions in the spleen.

During the first 3 days of the patient's hospitalization, his temperature rose above 38°C once (when blood samples were taken for two sets of blood cultures). He then discharged himself from the hospital but was readmitted 3 days later with a fever (temperature of 40°C) and an erythematous, tender, warm lesion (10×14 cm in diameter) over his left ankle. Blood samples were again drawn for culture, a presumptive diagnosis of cellulitis was made, and iv cefazolin (1 g t.i.d.) was prescribed.

On the following day, bullae formed over the lesion and the patient developed several round, erythematous papules (0.5–2 cm in diameter) that were scattered over his abdomen and all four extremities. The bullae were aspirated, and specimens were cultured. Direct examination of gram-stained aspirates revealed gram-negative rods. The antibiotic regimen was changed to ceftizoxime (2 g iv b.i.d.) and amikacin (15 mg/[kg · d]), but his general condition rapidly deteriorated over the next few hours (his temperature was 35.6°C, his respiratory rate was 28, and his WBC count was $19{,}800 \times 10^9$/L). The patient was transferred to the intensive care unit, where he received measures for the management of sepsis including noninvasive ventilatory support.

Culture of the aspirate yielded a gram-negative, oxidase-negative, nonfermentative rod. The isolate was resistant to cefazolin and ampicillin (zone diameters, 6 and 14 mm, respectively) and was susceptible to amoxicillin/clavulanate, amikacin, ciprofloxacin, and ceftizoxime (zone diameters of 24, 30, 30, and 30 mm, respectively). On day 14 after admission, the same organism was reported to have grown in the initial two blood culture bottles (these bottles contained tryptose agar and broth biphasic culture media), and blood cultures yielded this organism after the skin lesions appeared. The organism was identified as *F. oryzihabitans* (Vitek ID No. 606470001000).

The patient's condition improved within a few days, and central necrosis developed in the erythematous papular skin lesions. He made a full recovery, although skin grafting was later required because of soft-tissue necrosis over his left ankle. The patient received ceftizoxime therapy for 3 weeks and amikacin therapy for 2 weeks followed by ofloxacin (400 mg b.i.d. orally for an additional 4 weeks). An abdominal CT was performed on hospital day 18, when the number and size of the splenic lesions were reduced. His blood glucose level was well regulated, and he was discharged from the hospital and continued to receive therapy with oral antidiabetics. The patient was followed up for $1\frac{1}{2}$ years, and no other underlying disorder was seen. A follow-up CT performed 1 year after discharge revealed that the splenic lesions had disappeared.

To our knowledge, we report the first case of *F. oryzihabitans* infection in a patient who presented with splenic abscesses and whose sole risk factor was diabetes. *F. oryzihabitans* is an infrequent clinical isolate and an uncommon cause of sepsis. Two cases of community-acquired soft-tissue infection caused by *F. oryzihabitans* have been recently reported [3], but most of these infections occur in immunocompromised patients with central venous catheters in situ [4].

Although *F. oryzihabitans* infection is predominantly seen in immunocompromised patients who receive therapy with multiple antimicrobials (which can lead to the emergence of resistant organisms), *F. oryzihabitans* remains susceptible to most agents used to treat these patients [5]. Lucas et al. reported that most patients with sepsis due to *F. oryzihabitans* may be successfully treated with a combination of antipseudomonal β-lactam agents and aminoglycosides given for a mean of 14 days (range, 9–29 days) [4].

After our patient received a 3-week course of iv therapy, we chose to continue therapy with an oral fluoroquinolone for an additional 3 weeks because of the lack of information related to *F. oryzihabitans* sepsis accompanied by splenic abscesses.

It is unclear how this patient was exposed to *F. oryzihabitans.* Although *F. oryzihabitans* was once considered not to be a clinically significant pathogen in humans and was later reported to be capable of causing disease (particularly in immunocompromised patients), this case and recent data suggest that this organism should be considered as capable of causing community-acquired infections, including splenic abscesses and sepsis.

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Pseudomonas stutzeri Community-Acquired Pneumonia Associated with Empyema: Case Report and Review

Pseudomonas stutzeri is a gram-negative, nonfermenting rod that rarely causes human infections. We report a case of community-acquired pneumonia (CAP) in a 62-year-old woman that was associated with empyema and bacteremia. To our knowledge, this is the fifth reported case of CAP and the first case associated with empyema due to this organism.

A 62-year-old woman with chronic liver disease due to hepatitis C was admitted to the emergency department because of fever, a productive cough, and pleuritic chest pain of 1 week's duration. She was afebrile, and her vital signs were as follows: blood pressure, 110/70 mm Hg; pulse, 116 beats/minute; and respirations, 28/minute. Examination of the lungs was consistent with a right pleural effusion.

Laboratory tests revealed the following values: hemoglobin, 12.4 g/dL; hematocrit, 39.3%; WBC count, 41,100/mm^3 (91.4% granulocytes); platelet count, 184,000/mm^3; serum creatinine, 1.68 mg/dL; glucose, 361 mg/dL; and total protein, 8.37 g/dL. Basal arterial blood gases revealed a pH of 7.18, a Pa_{O_2} of 72.9 mm Hg, a Pa_{CO_2} of 24 mm Hg, and an H_{CO_3} of 8.8 mEq/L. A chest roentgenogram revealed right-lower-lobe pneumonia with a pleural effusion. Thoracocentesis yielded an exudate with a pH of 6.76, a glucose level of 189 mg/dL, a protein level of 4.78 g/dL, and a WBC count of 7,137/mm^3 (90% polymorphonuclear leukocytes), and gram staining did not reveal any organisms.

Blood was drawn for culture in the emergency department, and iv therapy with cefotaxime (2 g every 8 hours) plus tobramycin (100 mg every 12 hours depending on serum levels) was started; in addition, pleural drainage was done. The next day, therapy with cefotaxime was changed to that with ceftazidime (1 g every 24 hours). Since the patient went into septic shock and her mental status changed, she was hospitalized in the intensive care unit, where she experienced multiorgan failure. Orotracheal intubation plus mechanical ventilation was initiated, and the patient received therapy with catecholamines and repeated transfusions of RBCs, plasma, and platelets; in addition, she underwent peritoneal dialysis.

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The following day, the two admission blood cultures as well as cultures of pleural fluid yielded a gram-negative nonfermentative rod that was later identified as *P. stutzeri.* Since the patient remained afebrile, treatment was not changed once the isolate's susceptibility to ceftazidime and tobramycin was determined. Blood cultures performed during antibiotic treatment were negative, as were cultures performed on day 24 of hospitalization, so antibiotic therapy was discontinued. Eleven days later the patient began hemodialysis because of an episode of peritonitis related to peritoneal dialysis; she continued to undergo hemodialysis until diuresis was noted. Although she was discharged after 72 days of hospitalization because her symptoms had disappeared, the chest roentgenogram showed a left pleural effusion.

One month later, the patient was readmitted because of anemia (hemoglobin, 8.4 g/dL), severe malnutrition, and persistence of the left pleural effusion. The results of all laboratory tests were normal, and cultures and cytologic examination of the pleural fluid were negative. The patient was discharged after she partially recovered. One year later, the pleural effusion had resolved and her nutritional status had improved.

The *P. stutzeri* isolate was susceptible to piperacillin, ticarcillin/clavulanic acid, third-generation cephalosporins, imipenem, aztreonam, norfloxacin, ciprofloxacin, and aminoglycosides and was resistant to ampicillin, amoxicillin/clavulanic acid, cephalothin, cefazolin, cefoxitin, cefuroxime, and co-trimoxazole.

To date, four cases of community-acquired pneumonia due to *P. stutzeri* have been reported (table 1); complete data are available for three of them. We report the fifth case of this infection. The cases, including our report, occurred in three males and one female, and the median age was 57 years (range, 40–70 years). The clinical presentation was typical of CAP in two cases. In our case, the patient was afebrile at the time of admission although she had had a temperature of up to 39°C the previous week. The mean WBC count in all four cases was 18,325/mm^3 (range, 5,500–41,100/mm^3). The chest roentgenogram showed a lower-lobe infiltrate in two cases (one in the right lower lobe, one in the left), a right-upper-lobe infiltrate in one case, and a bilateral alveolar diffuse infiltrate in one case.

A pleural effusion was noted only in our patient's case, and culture of pleural fluid yielded *P. stutzeri* in only our case. On admission, patients 3 and 5 were critically ill and had severe respiratory failure that required orotracheal intubation and mechanical ventilation as well as therapy with catecholamines. Patients 1 and 5 had renal failure and required hemodialysis and peritoneal dialysis, respectively. Patient 5 also required transfusions. The four isolates of *P. stutzeri* were susceptible to third-generation cephalosporins, imipenem, and aminoglycosides.

Table 1. Summary of data from five cases of *Pseudomonas stutzeri* community-acquired pneumonia (including the present report).

Case no.	Year [reference]	Age (y)/ sex	Underlying conditions and/ or risk factors	Source of *P. stutzeri*	Clinical presentation	Antibiotic therapy	Outcome
1	1987 [1]	70/M	Multiple myeloma, cytotoxic therapy	Two blood cultures	Pneumonia	Ampicillin + gentamicin	Satisfactory
2	1992 [2]	56/M	Smoking, alcoholism, squamous cell carcinoma	Transthoracic needle aspirate	Pneumonia	Ceftazidime	Satisfactory
3	1992 [2]	40/M	Chronic liver disease, alcoholism	Bronchial brushing	Pneumonia	Imipenem + amikacin	Death
4	1993 [3]	?	Chronic obstructive pulmonary disease	Transtracheal aspirate	Pneumonia	?	Satisfactory
5	1997 [PR]	62/F	Chronic liver disease	Two blood cultures and two pleural fluid specimens	Pneumonia and empyema	Ceftazidime + tobramycin	Cured

NOTE. PR = present report.

P. stutzeri is a saprophyte found in soil and water, and it has been rarely involved in human infections such as wound infections, septic arthritis, eye infections, otitis media, CAP, and bacteremia [4, 5]. Gram-negative bacilli cause 9%–20% of CAPs, and it is believed that infection is due to aspiration of the pathogen from the oropharynx. Diabetics and alcoholics are more frequently colonized by these organisms [6]. Moreover, pneumonia is more common among older patients and those with coexisting illnesses such as chronic obstructive pulmonary disease and chronic liver disease [7]. In fact, all five patients with *P. stutzeri* CAP had an underlying illness or a risk factor that predisposed them to pneumonia or were debilitated.

Despite the low virulence of *P. stutzeri,* two patients had a severe illness that required admission to the intensive care unit. Etiological diagnosis of pneumonia due to gram-negative bacilli is difficult since blood cultures are usually sterile, empyema is only an occasional complication, and it is not always possible to perform invasive techniques. Expectorated specimens are often contaminated with aerobic gram-negative bacilli that colonize the oropharynx; gram staining of sputum specimens is suggestive only of the etiology of infection [6]. In our case, *P. stutzeri* was undoubtedly the etiological agent as it was isolated from two blood cultures and pleural fluid. *P. stutzeri* is usually susceptible in vitro to third-generation cephalosporins, carbapenems, monobactams, aminoglycosides, antipseudomonal penicillins, and trimethoprim-sulfamethoxazole, and it is variably susceptible to ampicillin [5].

To our knowledge, we report the first case of empyema caused by *P. stutzeri* and the fifth case of CAP due to *P. stutzeri.* Because of this organism's low virulence, patients who develop CAP must have a predisposing condition. The etiological role of *P. stutzeri* in CAP must be confirmed because infections caused by this organism are rare and because it is usually considered to be a saprophyte.

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Necrotizing Fasciitis Caused by Unencapsulated *Haemophilus influenzae*

Noninvasive strains of *Haemophilus influenzae* usually cause mucosal infections, whereas invasive strains cause meningitis, septic arthritis, and cellutis. Neither strains are commonly implicated as a cause of necrotizing fasciitis [1]. We report a case of necrotizing fasciitis due to unencapsulated *H. influenzae.* A 45-year-old previously healthy insulin-dependent diabetic with a recent history of untreated and asymptomatic monoclonal gammopathy of unknown significance bruised his right buttock in a skiing accident in February 1993. The patient had also experienced symptoms of chronic bronchitis all winter and early spring. The gluteal tenderness had almost subsided when he felt a sudden sharp pain in his right trochanteric region while playing tennis in May 1993. An orthopedic surgeon diagnosed acute bursitis of the greater trochanter and prescribed an oral nonsteroidal antiinflammatory drug that temporarily relieved his pain.

The day after he began receiving the antiinflammatory drug, fever developed and the patient was admitted to the hospital; the right gluteal region and lateral thigh were found to be extremely tender. Laboratory studies revealed that muscle enzymes were not elevated, and an MRI scan revealed no abnormality except minimal subcutaneous edema. Although antibiotic therapy with amoxicillin/clavulanate, gentamicin, and metronidazole was immediately initiated, generalized swelling of the entire thigh developed (the skin appeared normal, and crepitus was not noted). Blood cultures were repeatedly negative, and histological examination of specimens obtained from the thigh muscle by a needle biopsy did not reveal any organisms.

The patient became increasingly septicemic despite receiving cephalosporin therapy, and on day 17 after admission a repeated MRI scan showed extensive swelling and liquification of the tissues surrounding the musculus gluteus medius, musculus vastus lateralis, and the abductor muscles. The adjacent joints and the muscle itself appeared normal.

Aggressive surgical incision and debridement of all involved regions revealed a yellowish material that contained numerous WBCs. Gram staining and culture of the purulent material revealed an unencapsulated strain of *H. influenzae* that was susceptible to ciprofloxacin and imipenem but no other organism; a large number of organisms were noted. Culture of subsequently obtained tracheal secretions revealed the same strain of *H. influenzae,* but culture of blood did not. The wound was packed open, the fascial planes were debrided daily, and the dressings were changed frequently until healthy granulation appeared. Therapy with ciprofloxacin was given intravenously for 2 weeks, and the patient recovered fully except for a residual limp.

It is of particular interest that in our case the necrotizing fasciitis was caused exclusively by a noninvasive strain of *H. influenzae.* The only reported case in which *H. influenzae* type b was associated with necrotizing fasciitis occurred in a 13-month-old infant [2]. Necrotizing fasciitis commonly occurs as a consequence of major or minor trauma in debilitated patients and is typically caused by polymicrobial synergistic infections (e.g., those due to staphylococci, hemolytic non-group A streptococci, anaerobic bacteria, enterobacteria, and clostridia). The clinical presentation of necrotizing fasciitis can be acute or subacute, the muscle and skin are usually not affected, and a minority of cases proceed to toxic shock [3].

Our patient was debilitated by his long-standing (31 years) diabetes mellitus, which was poorly controlled metabolically (glycosylated hemoglobin A1c, 10%) and caused local microcirculatory and defense impediments, and by the monoclonal gammopathy of unknown significance that compromised humoral defense mechanisms in general. Soft-tissue invasion by *H. influenzae* in this immunocompromised host may have occurred via hematogenous spread from the upper respiratory tract to the local tissues, which were rendered susceptible by the skiing and tennis injuries and the diabetic tissue alterations. The clinical course of soft-tissue infections caused by unencapsulated *H. influenzae* is subacute, which is probably due to the fact that the bacterium is primarily noninvasive.

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Levels of Transforming Growth Factor β1, Tumor Necrosis Factor α, and Interleukin 6 in Cerebrospinal Fluid: Association with Clinical Outcome for Children with Bacterial Meningitis

Transforming growth factor β1 (TGF-β1) has broad activity in the modulation of the immune response. TGF-β1 may modulate the inflammatory response through its inhibition of cytokine production, including that of TNF-α, IL-1β, IL-6, and IFN-γ [1–5]. The antiinflammatory effect of TGF-β has been shown in experimental meningitis [6].

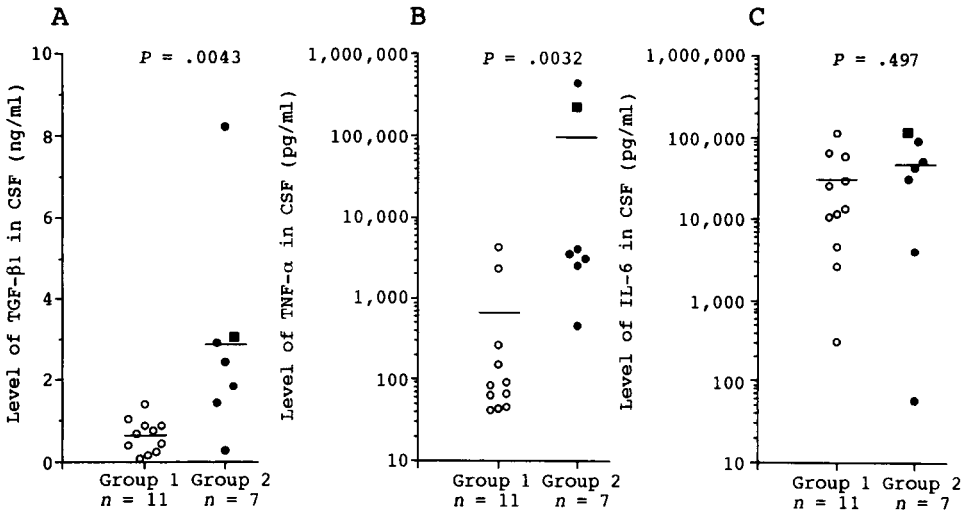

Figure 1. CSF levels of TGF-β1 (*A*), TNF-α (*B*), and IL-6 (*C*) in children with bacterial meningitis. Group 1 = children who survived. Group 2 = children who died or had sequelae. Mean values are indicated by horizontal lines. ■ = an 8-day-old neonate with bacterial meningitis due to *Proteus* species who died 1 day later.

In the present study, we measured levels of TGF-β1, TNF-α, and IL-6 in CSF from children with bacterial meningitis. CSF samples were obtained from 18 children with bacterial meningitis (6 males and 12 females; age range, 8 days to 9 years; mean age, 1.6 years) and 14 afebrile children without neurological sequelae who did not have pleocytosis or meningitis and who served as controls (seven males and seven females; age range, 8 months to 14 years; mean age, 4.7 years) who were admitted to our hospital between 1984 and 1995. We divided the children with bacterial meningitis into two groups, i.e., those who survived (Group 1, $n = 11$) and those who died or had neurological sequelae (Group 2, $n = 7$).

The day of onset of fever was considered as the first day of illness. Pretreatment CSF samples were taken from patients with meningitis to determine the levels of cytokines on days 1–4 (mean ± SD, 2.6 ± 1.2 days) of illness. CSF cultures from patients with bacterial meningitis yielded *Haemophilus influenzae* ($n = 11$), *Streptococcus pneumoniae* ($n = 4$), group B streptococci ($n = 1$), *Proteus* species ($n = 1$), and *Listeria monocytogenes* ($n = 1$). The patients with bacterial meningitis were treated with multiple antibiotics that were effective for these bacteria. No patients were treated with steroids.

The concentrations of TGF-β1, TNF-α, and IL-6 in CSF were determined with ELISA kits (TGF-β1, Genzyme; TNF-α and IL-6, R & D Systems). The assay had detection limits of 0.05 ng/mL for TGF-β1, 39 pg/mL for TNF-α, and 31.2 pg/mL for IL-6. All values were given as mean ± SD. The differences in the results between groups were analyzed with use of the Mann-Whitney *U* test. Correlations were analyzed with use of Spearman's rank correlation coefficient test.

The mean (±SD) level of TGF-β1 in the CSF of the controls was 0.19 ± 0.11 ng/mL. The TNF-α and IL-6 levels of CSF in the controls were all below the detection limits (<39 pg/mL and <31.2 pg/mL, respectively). The TGF-β1, TNF-α, and IL-6 levels of CSF in the patients were significantly higher than those in the controls ($P = .0003$, $P < .0001$, and $P < .0001$, respectively). The TGF-β1 and TNF-α levels of CSF in Group 2 were significantly higher than those in Group 1 ($P = .0043$ and $P = .0032$, respectively) (figure 1). There was a positive correlation between TGF-β1 and TNF-α levels in the CSF of children with bacterial meningitis ($r = 0.83$, $P = .006$). We speculate that the high TGF-β1 level in CSF reflects the high TNF-α level in CSF in the patients with bacterial meningitis.

By considering the inhibitory effects of TGF-β1 on TNF-α production, we calculated the ratio of the TNF-α level to the TGF-β1 level in the CSF of groups 1 and 2. The ratio of TNF-α to TGF-β1 in Group 2 was significantly higher than the ratio of these cytokines in Group 1 ($P = .013$). It is therefore likely that the difference in levels between TNF-α and TGF-β1 may be related to the pathogenesis of the neurological sequelae.

Levels of the antiinflammatory cytokine IL-10 that were similar to the levels of TGF-β1 were elevated in CSF during the onset of bacterial meningitis [7]. The high TGF-β1 and IL-10 levels may inhibit inflammation because of the production of the proinflam-

Informed consent was obtained from the patients' parents, and the human experimentation guidelines of Yamaguchi University School of Medicine were followed in the conduct of this clinical research.

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matory cytokines. Our results suggest that both TGF-β1 and TNF-α levels in CSF are important immunologic parameters for determining neurological sequelae in children with bacterial meningitis.

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Increased Endothelin Levels in Cerebrospinal Fluid Samples from Adults with Bacterial Meningitis

The rates of morbidity and mortality associated with bacterial meningitis remain high, although antibiotic therapy for this infection has improved during recent decades [1, 2]. The unfavorable clinical outcome is often due to cerebrovascular complications that develop during the acute phase of the disease [3, 4]. Meningitis-associated ischemic complications are believed to be caused by an inflammatory infiltration of vessel walls (vasculitis), by encroachment of vessels by the inflammatory exudate, and/or by vasospasm [3, 5, 6]. The pathophysiology of cerebral vasospasm during bacterial meningitis is still unclear.

Experimental and clinical data support the potential involvement of endothelins (ETs) in the pathophysiology of cerebral ischemia and vasospasm following subarachnoid hemorrhage. Thus, ETs are potent and long-lasting vasoconstrictors of large and small cerebral arteries in vivo and in vitro [7]. In addition, intracerebroventricular and intracisternal injection of ET-1 causes vasoconstriction leading to ischemic cerebral lesions in the rat [7]. The aim of our study was to investigate whether ETs are possible mediators of cerebrovascular complications in patients with bacterial meningitis.

We measured ET levels in 30 CSF samples from 15 adult patients (eight women and seven men, aged 19–76 years [median age, 51 years]) with bacterial meningitis. Meningitis was caused by the following bacteria: *Streptococcus pneumoniae* ($n = 8$), *Neisseria meningitidis* ($n = 2$), *Haemophilus influenzae* ($n = 2$), *Escherichia coli* ($n = 1$), and *Streptococcus bovis* ($n = 1$). No bacteria were detected in the CSF of one patient with purulent meningitis ($n = 1$). CSF samples from 10 HIV-infected patients and 10 patients with noninflammatory neurological disorders served as controls.

Since astrocytes have been reported to be a major source of ETs in experimental cerebral ischemia [8, 9], we investigated whether primary rat astrocytes can be induced to release ETs upon stimulation with pneumococci. Primary astrocyte cultures were prepared from the cerebral cortex of neonatal Wistar rats, as described previously [10]. ET concentrations in the cell culture medium were measured 48 hours after stimulation. The following experimental groups were investigated: primary rat astrocytes stimulated with heat-killed pneumococci (HKP; 10^7 cfu/mL) that were untreated or treated with phosphoramidon, an ET-converting enzyme inhibitor (100 μM); treated with cycloheximide (20 μM); treated with actinomycin D (1 μM); treated with BQ-123, a selective ET_A receptor antagonist (1 μM); and treated with BQ-788, a selective ET_B receptor antagonist (10 μM). Supernatants of astrocyte cultures that had not been stimulated with HKP were investigated as controls. ET concentrations in CSF and in supernatants of astrocyte cultures were determined by a commercially available EIA (Biomedica GmBH, Vienna, Austria). This EIA is highly sensitive for ET-1 and ET-2, showing only low cross-reactivity with ET-3 (<5%) and Big-ET (<1%). To estimate the amounts of ET, a standard curve was constructed by using known concentrations of ET standard dissolved in normal human CSF and in cell culture medium. ET concentrations in samples were expressed as pM. The detection limit of the assay was 0.1 pM.

At the time of admission (within the first 48 hours after the onset of neurological disease), ET concentrations were significantly elevated in the CSF samples from patients with bacterial meningitis, as compared with samples from both HIV-infected patients and patients with noninflammatory neurological disorders (table 1). There was no significant correlation between ET levels and CSF WBC counts or CSF protein levels. Follow-up determinations (performed for 10 patients 2–7 days after the onset of disease and for eight patients 7–60 days after onset of disease) revealed a significant decrease in ET concentrations in the CSF samples from patients with bacterial meningitis (table 1).

In astrocyte cultures, stimulation with HKP induced a threefold increase in ET levels over those observed in cell culture superna-

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Table 1. Endothelin levels, WBC counts, and protein levels in the CSF of adults with bacterial meningitis, HIV infection, and noninflammatory neurological disorders.

CSF parameter	Patients with bacterial meningitis for whom indicated parameter was measured <1 d after onset of disease (n = 12)	Patients with bacterial meningitis for whom indicated parameter was measured 2–7 d after onset of disease (n = 10)	Patients with bacterial meningitis for whom indicated parameter was measured >7 d after onset of disease (n = 8)	Patients with HIV infection (n = 10)	Patients with other neurological disorders (n = 10)
Mean ET level (pM) ± SE	1.93 ± 0.65	0.29 ± 0.08*	0.19 ± 0.05*	0.11 ± 0.06†	0.15 ± 0.06†
Mean WBC count (/mm^3) ± SE	4,705 ± 2,018	1,043 ± 423	187 ± 94*	7 ± 3†	2 ± 1†
Mean protein level (mg/dL) ± SE	388 ± 78	157 ± 40*	101 ± 24*	65 ± 17†	43 ± 4†

NOTE. ET = endothelin.

* With use of the paired Student's t test, $P < .05$ when the ET levels in these patients were compared to ET levels in a patient with bacterial meningitis on admission. P values were corrected for repeated measures by using the Bonferroni-Holm procedure.

† With use of analysis of variance and Scheffe's test, $P < .05$ when the ET levels in these patients were compared to ET levels in patients with bacterial meningitis on admission.

tants that were not stimulated with HKP (0.44 ± 0.04 pM vs. 0.15 ± 0.03 pM; $P < .05$; analysis of variance [ANOVA] and Scheffe's test). Treatment with phosphoramidon, cycloheximide, or actinomycin D prevented the increase in ET concentrations (0.13 ± 0.03 pM, below the detection limit, and below the detection limit, respectively) ($P < .5$ by ANOVA and Scheffe's test compared with untreated, HKP-stimulated astrocytes). In addition, administration of the ET_A receptor antagonist BQ-123 inhibited ET production, whereas treatment with the ET_B receptor antagonist BQ-788 augmented ET levels in cell culture supernatants (0.11 ± 0.03 pM and 1.62 ± 0.29 pM, respectively; $P < .05$ compared to untreated, stimulated astrocytes [ANOVA and Scheffe's test]). These findings may be explained by an autocrine-signaling mode for ETs in astrocytes. Recent studies have provided evidence that ETs may regulate their own release in an autocrine fashion in a variety of cell types (e.g., endothelial cells or astrocytes) [7, 11].

Our results show that ET concentrations are elevated in CSF during the acute stage of bacterial meningitis and that astrocytes are a possible source of ET production. Thus, ET is a potential candidate for mediating meningitis-associated cerebral hypoperfusion (as has been shown in studies with use of single photon emission computed tomography [12]) and brain infarction [5]. Further clinical and experimental studies, such as correlation of ET concentrations in the CSF with the results of transcranial Doppler ultrasonography and MRI, are required to clarify the role of ETs in the pathophysiology of cerebrovascular complications in patients with bacterial meningitis.

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Apophysomyces elegans Limb Infection with a Favorable Outcome: Case Report and Review

Apophysomyces elegans is a saprophytic soil fungus of the class Zygomycetes and the order Mucorales, which is distributed worldwide [1, 2].

A. elegans is able to progressively invade tissues in healthy and immunocompromised hosts; this invasion apparently begins at the site of a prior injury such as a trauma, a burn, or invasive procedures [1–3]. We report, to our knowledge, the first case of limb infection caused by *A. elegans* in an immunocompetent patient from Venezuela; the patient was treated successfully without amputation.

A 34-year-old previously healthy male who lived in a rural area 45 miles from Caracas sustained a traumatic wound on his right thigh, which was produced by a soiled weedhook on 17 December 1994. On admission, the patient was severely ill, and necrosis of the skin, subcutaneous fat, and muscles in the area of the wound was noted. Some necrotic areas were covered by a whitish mold-like growth; therefore, empirical treatment with amphotericin B and fluconazole was started.

Progressive necrosis and new cotton-like lesions appeared, and the patient underwent surgery on 2 January. Biopsy of the resected tissue showed necrotizing cellulitis caused by a zygomycete, and oral itraconazole (200 mg/d) was substituted for fluconazole in the treatment regimen. The local wounds began to heal, and the remaining necrotic tissue was removed on 9 January. The large blood vessels of the right thigh were exposed.

On 10 January, the culture was reported as being positive for *A. elegans* (figure 1). The patient's condition improved, but on 10 February, severe right femoral artery bleeding developed. A Fogarty catheter was passed through the artery for removal of clots and surgical repair of the artery. On 25 February a second episode of bleeding from the superficial femoral artery occurred. The bleeding was stopped by covering the vessel with muscle. There were no further complications. After 1 month, grafts were placed on the thigh, and amphotericin B was administered (total dose, 3.5 g). Itraconazole was withdrawn after 8 weeks. The patient was discharged in excellent condition in April 1995. In June 1995, his graft and wounds had healed, with no evidence of recurrent fungal infection.

A. elegans is able to invade the subcutaneous tissue, causing necrosis of fat and the superficial fascia. Further spread may produce massive muscle necrosis, destruction of blood vessel walls with subsequent thrombosis, hemorrhagic infarction, and mycotic embolization to distant tissues. In our immunocompetent patient, this fungus produced progressive necrosis. We believe the portal of entry for the infection was the traumatic wound involving the skin and subcutaneous tissues [1].

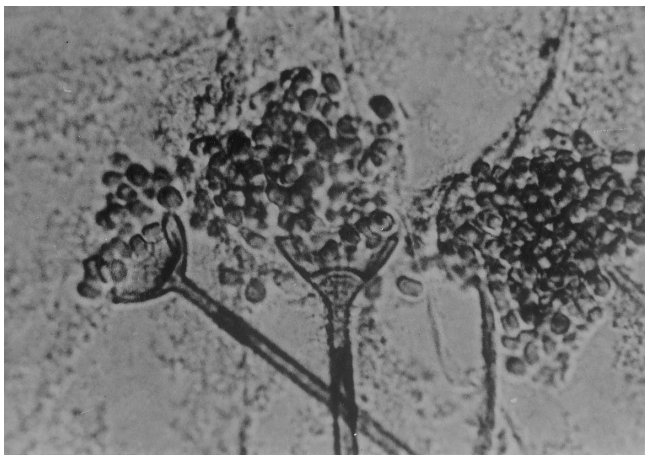

Figure 1. Ruptured mature sporangia of *Apophysomyces elegans,* grown on lactrimel agar, showing distinctive fan-shaped and well-developed dark apophyses and oblong-to-subglobose sporangiospores. (Stain, trypan blue lactophenol; original magnification, ×800).

A. elegans was first described by Misra et al. in 1979 [4]. In 1982, *A. elegans* was isolated from the bronchial washings of a patient in the United States [5]. In our case, *A. elegans* grew well on Sabouraud dextrose agar at a temperature ranging from >37°C to 40°C but failed to sporulate on Sabouraud dextrose agar. After 1 week, the fungus sporulated on lactrimel and sablac agars at room temperature (23–28°C) [6].

We performed an extensive literature search and found 14 cases of *A. elegans* infection. Eleven of them had occurred in the United States, one in Australia, one in India, and one in Aruba [3, 7–10]. Most of the patients had infections in the extremities, and trauma or skin lesions were the portals of entry for the infections, except in the patient from India, who had undergone a postinguinal herniorrhaphy before onset of infection [7]. All but one of the patients (a female from the United States who had uncontrolled diabetes mellitus [3]) had been previously healthy.

Late diagnoses were common among these patients. They underwent extensive surgical debridement, and tissue necrosis was the common pathological finding. Most of these patients received high doses of amphotericin B, and some also received liposomal amphotericin B. Seven of the 14 patients had limb involvement. Two of these seven patients died, and three underwent amputation.

Early diagnosis is the key to an improved prognosis for patients with *A. elegans* infection. New antimycotic drugs and in vitro studies of susceptibility of this fungus to the new drugs are needed to provide a better initial therapeutic approach. Our patient represents the 15th case of *A. elegans* infection described in the worldwide literature and the first case from

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Latin America in an immunocompetent patient with a limb infection that was cured without amputation.

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Measurement of Human Immunodeficiency Virus (HIV) Type 1 RNA Load Distinguishes Progressive Infection from Nonprogressive HIV-1 Infection in Men and Women

The HIV-1 load in plasma or serum has been found to correlate with disease progression in men as well as response to antiviral therapy and transmission of HIV-1 from mother to child [1–7]. Because the worldwide incidence of HIV-1 infection among women, including those in the United States, has increased dramatically in recent years, studies of viral load need to include women as well as men to determine if the relationship between HIV-1 load and disease progression applies to both genders. To investigate distinctive patterns of HIV-1 disease progression and their relationship to viral load in both women and men, we used reverse transcription quantitative competitive PCR (RT QC-PCR) to measure serum or plasma HIV-1 RNA levels in four groups of well-characterized HIV-1 infected patients. We retrospectively studied 45 patients, including 22 women and 23 men. These HIV-1-infected adults were stratified, on the basis of patterns of clinical HIV-1 disease progression, into the following four categories:

Category 1, long-term nonprogressive disease ($n = 7$). These patients had been infected at least 8 years and had $CD4^+$ cell counts of $>450/mm^3$ at the most recent measurement. None of these patients had received antiretroviral treatment.

Category 2, clinically stable nonprogressive disease ($n = 12$). These patients had $CD4^+$ cell counts of $200–450/mm^3$. Eleven of these patients had received antiretroviral treatment.

Category 3, progressive HIV-1-related disease ($n = 9$). The disease was considered progressive if the patient developed any of the following problems during the 1- to 2-year observation period after the blood was collected: an AIDS-defining opportunistic infection, wasting, or any other HIV-related clinical condition; p24 antigenemia; or a loss of >100 $CD4^+$ cells/mm^3. None of these patients had histories of opportunistic infections at the time the blood specimen was obtained. Eight patients had received antiretroviral treatment.

Category 4, a history of AIDS-defining opportunistic infections at the time the blood was collected ($n = 17$). Eleven of these patients were taking antiretroviral drugs.

Each of the patients in categories 1–3 were followed up clinically for at least 1–2 years after the specimen was obtained. Six women in category 4 (patients 40–45) were included in a substudy of the Womens Interagency HIV Study (WIHS) in the Bronx, New York, and they provided plasma. All other patients were patients at Long Island Jewish Medical Center (New Hyde Park, NY), and they provided serum. HIV-1 RNA was extracted from frozen serum or plasma and quantitated by means of RT QC-PCR with use of a modification of the technique of Piatak et al. [1, 6]. Correlations between $CD4^+$ cell count, HIV-1 RNA level, and disease category were studied by using the nonparametric Spearman correlation coefficient and its P value.

We first analyzed how the HIV-1 RNA load related to the category of disease. When the four categories were analyzed statistically as a whole, we found that the mean HIV-1 RNA load increased with increasing category of disease progression (Spearman correlation = 0.73; $P = .0001$; figure 1). This correlation also pertained when the populations of men and women in each group were analyzed separately (Spearman correlation = 0.74; $P = .0001$ for men; Spearman correlation = 0.73; $P = .0001$ for women).

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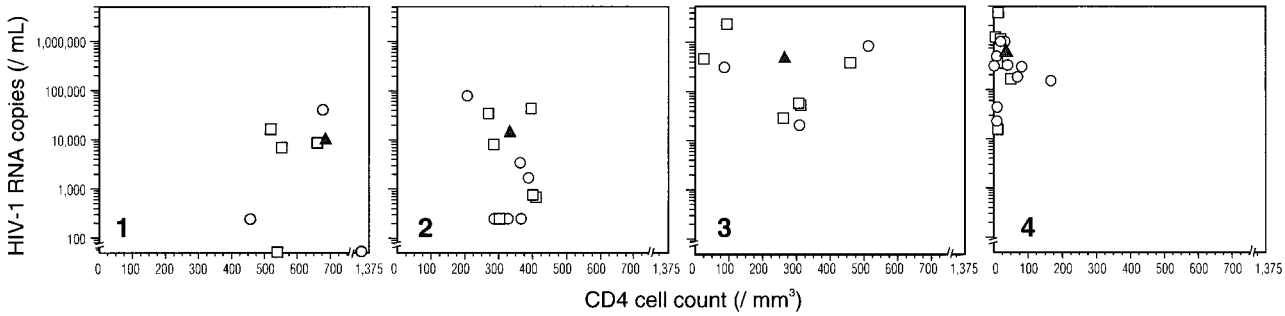

Figure 1. HIV-1 RNA levels and $CD4^+$ cell counts for patients in each category of disease progression (see categories 1–4 in text); ○ = individual women, □ = individual men, ▲ = mean values for men and women combined.

The most striking differences in viral loads were observed when the two groups of patients with progressive disease (categories 3 and 4) were compared with the groups who had nonprogressive disease (categories 1 and 2) (figure 1). HIV-1 RNA loads of <50,000 copies/mL were strongly correlated with nonprogressive disease, and HIV-1 RNA loads of >50,000 copies/mL were correlated with progressive disease. These relationships were statistically significant for men ($P < .0001$), women ($P < .01$), and the combined group of all patients ($P < .0001$, Fisher's exact test). There was a small fraction of patients in categories 3 and 4 who had low viral loads. Antiviral treatment may have contributed to the low RNA levels in some of these patients.

We found that the mean HIV-1 RNA load increased with decreasing $CD4^+$ cell counts in both men and women (Spearman correlation = −0.64; $P = .001$ for men; Spearman correlation = −0.60; $P = .003$ for women; Spearman correlation = −0.62; $P = .0001$ for men and women combined). However, the HIV-1 RNA load in most cases was an earlier predictor of disease progression than was the $CD4^+$ cell count.

There were no statistically significant differences between the genders in either viral load or $CD4^+$ cell count in any of the categories of disease progression (Wilcoxon rank sum test).

In this study, we found a strong correlation of viral load with disease progression for both men and women; this correlation was statistically significant for both men and women. These data are consistent with the concept that there may be HIV-1 RNA thresholds that play a role in determining clinical outcomes [6, 7]. Measurement of HIV-1 RNA appears to be clinically useful in predicting disease progression and monitoring the effectiveness of antiviral therapy in women as well as in men.

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Chromobacterium violaceum Infection of the Deep Neck Tissues in a Traveler to Thailand

Chromobacterium violaceum is frequently found in soil and water in tropical and subtropical regions, but rarely in temperate regions [1]. Infection often occurs after exposure of damaged skin to stagnant water or soil [2]. Progression to systemic infection is often rapid, with the development of multiple metastatic abscesses that involve the liver, spleen, lung, or skin [2–5]. We report, to our knowledge, the first case of *C. violaceum* infection in the deep tissues of the neck.

A previously healthy 27-year-old male presented after he developed fever, pharyngitis, and odynophagia while traveling in Thailand. One week earlier he had cut his left leg on coral. A pustule developed adjacent to the wound 4 days before the onset of his illness. He was admitted to the hospital and received a 6-day course of antibiotics. His condition did not improve, and he underwent bilateral tonsillectomy for presumed quinsy. He was discharged with instructions to take ofloxacin (100 mg b.i.d.) and amoxicillin/clavulanic acid (500 mg/125 mg t.i.d.), and he returned to New Zealand.

When he was examined 1 day later, bilateral tonsillar exudate was present, but he was well, and the antibiotic therapy was stopped. Eight days later he was readmitted to the hospital with fevers and increasing left-sided neck pain. His temperature was 37.5°C. He had bilateral tonsillar exudates. There was local tenderness over the bony origin of the sternomastoid. Findings on physical examination were otherwise normal.

Laboratory investigations showed a hemoglobin level of 94 g/L, a WBC count of 9.9×10^9/L, and cholestatic derangement of his liver enzymes; serology for HIV was negative. Blood cultures were sterile.

A diagnosis of retropharyngeal infection was made, and treatment with iv amoxicillin/clavulanic acid (1.0/0.2 g t.i.d.) was started. A neck CT scan did not show any fluid collection. The left internal jugular vein was nonenhancing and was presumed to be occluded. The patient remained unwell, with temperature spikes to 40°C. He developed pleuritic chest pain. A repeated CT scan on hospital day 5 showed a left prevertebral fluid collection and an opaque mastoid. Emergent left-jugular-vein ligation and excision, mastoidectomy, thrombectomy from the sigmoid sinus and jugular bulb, and drainage of the prevertebral abscess were performed.

Within 36 hours, *C. violaceum* was isolated from mastoid tissue and tracheal aspirates. The young colonies were smooth, convex, and 1 mm in diameter. The older colonies were β-hemolytic. The isolate did not decarboxylate either lysine or ornithine but hydrolyzed arginine. The Baxter Microscan identification system (Baxter Laboratories, West Sacramento, CA) gave a biotype number of 20355370405–150, which identified the isolate as *C. violaceum;* in addition, it was noted that the isolate's purple pigment production did not diffuse into clear nutrient agar plates. Disk susceptibility testing showed that the isolate was resistant to amoxicillin, cefuroxime, cefoxitin, ceftazidime, piperacillin, and ticarcillin but susceptible to chloramphenicol, gentamicin, amikacin, cotrimoxazole, tetracycline, imipenem, and ciprofloxacin. The MICs of gentamicin, amikacin, and ciprofloxacin were 2.0 mg/L, 16 mg/L, and <0.03 mg/L, respectively.

After the isolate was identified, iv therapy with amikacin (500 mg q8h) and chloramphenicol (1.2 g q8h) was begun. The patient initially remained grievously ill; there was radiographic evidence of septic emboli to his liver, and metastatic lesions were noted on his skin. However, by day 13, 6 days after the operation, his condition had clearly improved. The antibiotic regimen was changed to iv ciprofloxacin (200 mg q12h). Treatment with oral ciprofloxacin (750 mg b.i.d.) was started on day 17. He was discharged after 20 days. He completed 3 months of treatment and remains well 12 months later.

Systemic infection with *C. violaceum* is rare, and we did not consider this organism in the differential diagnosis for our patient. This is the first reported case of *C. violaceum* infection of the deep tissue of the neck. Our management of this case was complicated by the unusual spread of the infection; while quinsy can occasionally spread to the adjacent parapharyngeal space, spread to the prevertebral space is extremely rare. The focus of this infection was in the prevertebral space and adjacent skull base, with secondary effects on the mastoid and sigmoid-jugular system. Deep neck infections are associated with significant morbidity and mortality [6, 7], and the development of jugular thrombophlebitis with septic pulmonary emboli is a well-known complication [6]. Presumably, hematogenous seeding to the deep-neck-tissue spaces occurred in our patient some time after the infection developed on his leg.

Mortality rates of 60% have been described for patients with *C. violaceum* infection [5]. The optimal antibiotic regimen (the drug, mode of administration, or duration of administration) is not known. Aminoglycosides [2–4, 8] were used in four of the five survivors of *C. violaceum* infection for whom the antibiotic regimens were described. However, the MICs of gentamicin reported for three isolates [5, 8] and herein ranged from 1 mg/L to 5 mg/L. Clearly, *C. violaceum* is not extremely susceptible to gentamicin; our isolate was similarly not extremely susceptible to amikacin. These laboratory data cast doubts on the routine recommendation of aminoglycosides for this infection. Recently, because of relapses [2, 3], oral therapy has been given for 2 to 3 months after initial treatment with iv ciprofloxacin.

A quinolone and ceftazidime [9], with or without an aminoglycoside, could be initial therapeutic choices for patients from tropical regions who present with fulminant sepsis; *C. violaceum, Burkholderia pseudomallei,* and the usual bacterial pathogens would be covered by such a regimen.

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Clinical and Pathophysiological Aspects of Immune Complex Glomerulonephritis Associated with *Entamoeba histolytica* Abscess of the Liver

Various bacterial or parasitic infections are associated with glomerulonephritis. Detection of microbial antigens within glomerular lesions during the course of infection-associated glomerulonephritis suggests a direct link between the infectious process and glomerular damage. We describe a patient who presented with an amebic liver abscess in association with proliferative glomerulonephritis; ''humplike'' deposits were present in some glomeruli.

Fifteen days after a trip to Yemen, a 62-year-old woman presented to our hospital with fever and a pain in the left hypochondrium. Physical examination showed an enlarged liver. Laboratory values were as follows: erythrocyte sedimentation rate, 118 mm after 1 hour; hemoglobin level, 8.9 g/dL; WBC count, 10,660/mm^3; serum creatinine level, 1.1 mg/dL; serum urea level, 124 mg/dL; and levels of alkaline phosphatase and transaminases, twice the normal values. An abdominal ultrasonogram showed an intrahepatic image compatible with an 11-cm abscess. An EIA with *Entamoeba histolytica* antigen and hemagglutination was strongly positive (titers, 1,350 and 1,024, respectively), confirming the diagnosis of amebic liver abscess. Treatment with intravenous metronidazole (500 mg three times daily) and oral tilbroquinol (1 g/d) for 10 days was initiated, and within 4 days, the fever and abdominal pain had attenuated. The abscess was punctured on day 8 to prevent its spontaneous rupture. This procedure provided complete relief of the patient's symptoms.

The patient developed mild, generalized edema 3 days after treatment was started. Laboratory studies showed the following values: albumin concentration 23 g/L; serum creatinine level, 1 mg/dL; serum urea level, 82 mg/dL; and proteinuria, 5.8 g/d without leukocyturia or hematuria. Type 2 cryoglobulinemia was detected (monoclonal IgG-κ level, 0.23 g/L). No circulating immune complexes were detected. The levels of total hemolytic complement, C3, C4, and B were normal. Findings on an ultrasonogram of the kidneys and urinary tract were normal. A renal biopsy was performed, and examination of the glomeruli showed mesangial hypercellularity and presence of polymorphonuclear cells. Some glomeruli showed endomembranous and extramembranous ''hump-like'' deposits. The interstitium and vessels were normal.

Direct immunofluorescence revealed granular deposits of IgG and C3 along the glomerular basement membranes and in the mesangium. Electron microscopic investigation showed mesangial proliferation and mesangial, endomembranous, and extramembranous humplike deposits (figure 1). Attempts to detect the 170-kD immunodominant protein of *E. histolytica* with use of monoclonal antibodies yielded negative results. As these histopathological data were consistent with postinfectious glomerulonephritis, no treatment was prescribed. The renal abnormalities spontaneously disappeared within 2 months, the albumin concentration returned to normal, and the level of proteinuria decreased to 350 mg/d. At the last evaluation, the patient was free of renal symptoms.

In the present case, which resembles the case reported by Westendorp et al. [1], three findings suggested a causal link between the patient's visceral amebiasis and glomerulonephritis: the onset of glomerulonephritis during the course of the infection, the concomitant mixed cryoglobulinemia [2], and the presence of extramembranous humplike immune complex deposits. Microbial antigens can be found in glomerular immune complex deposits in patients with infection-associated glomerulopathies [3]. Immune complex deposition, which leads to the production of the C5b9 component (the so-called membrane attack complex [MAC]), results in glomerular basement membrane injury [4]. The inactivation of CD59, an inhibitor of C5b9, has been shown to worsen glomerular damage [5].

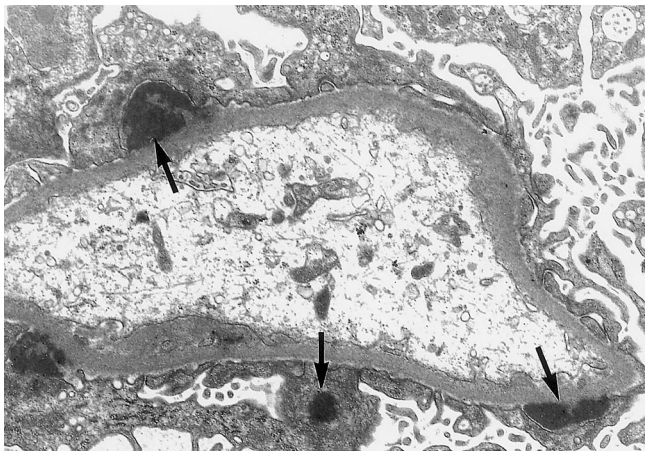

Figure 1. Transmission electromicroscopy shows extramembranous ''hump-like'' deposits (*arrows*) along the glomerular basement membrane in a patient with a liver abscess due to *Entamoeba histolytica* (original magnification, ×16,000).

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An immunodominant 170-kD protein of *E. histolytica* is considered to be essential for colonization and invasion of host tissues [6]. This protein is an adhesin and shares sequence identity with complement components and CD59 [7]. This protein is responsible for amebic resistance to complement by inhibiting the action of MAC on *E. histolytica* trophozoites. This protein is almost universally recognized by antibodies in the sera of patients with amebic liver abscesses, and it is possible that antibodies to the 170-kD protein play a role in glomerular injury. In fact, during amebic infection—especially once treatment is started—parasitic antigens are released and circulate in immune complexes (this possibly caused our patient's cryoglobulinemia). Glomerular immune complex deposition or in situ formation, possibly triggered by the lectin property of the 170-kD protein, could activate the complement cascade and lead to the formation of MAC. The cross-reactivity of antibodies to the 170-kD protein with CD59 could inactivate CD59 [7], thus favoring the deleterious effect of MAC on the glomerular basement membrane [5].

This sequence of events remains speculative, but studies on the nephrotoxic properties of amebic antigens—especially the 170-kD lectin—in experimental animal models could enlighten our understanding of the pathophysiology of glomerular injury during infection-associated glomerulonephritis.

In conclusion, as proteinuria is common during the course of visceral amebiasis [6], and renal biopsy is not routinely performed in areas where incidence of visceral amebiasis is high [3], glomerulonephritis may be a common, yet overlooked, complication of amebiasis. Physicians should be aware of this possible complication of amebic liver abscess, and we recommend repeated urinalyses for patients with amebiasis.

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Spontaneous Rupture of the Spleen Revealing Primary Human Immunodeficiency Virus Infection

Spontaneous rupture of the spleen has been described among patients with acute infections due to various microorganisms or with hematological diseases. We report a case of splenic rupture that occurred during acute HIV-1 infection.

A 60-year-old homosexual man was admitted to the hospital for evaluation of diarrhea, vomiting, a high fever (temperature, 100°F), and lethargy of 4 days' duration. On admission, physical examination revealed severe hypotension. The cervical lymph nodes were enlarged. The abdomen was moderately tender. Findings during the remainder of the physical examination were unremarkable. No history of trauma was recorded.

An abdominal ultrasonogram showed an intraperitoneal effusion. The hemoglobin level was 10.7 g/dL, the WBC count was 6,800/mm^3 with lymphopenia (lymphocyte count, 440/mm^3), and the platelet count was 45,000/mm^3. The prothrombin time was normal. Emergency laparotomy disclosed massive hemoperitoneum (1,500 mL of blood) that seemed to originate from the spleen. Splenectomy was performed.

On macroscopic examination, the spleen weighed 100 g, and a subcapsular hematoma was present. There were few microscopic lesions. The red pulp was congested. The red pulp cords were slightly hypercellular, and rare immunoblasts were intermingled with lymphocytes, plasma cells, neutrophils, and macrophages. The white pulp was slightly enlarged. The most striking feature was lymphoid infiltration of the subendothelial layer of the intrabecular veins.

Serological tests were negative for antibodies to *Toxoplasma gondii* and positive for IgG antibodies to cytomegalovirus and Epstein-Barr virus. On the day that the splenectomy was performed, an ELISA and western blot assay were negative for antibodies to HIV-1 and HIV-2, but a high level of p24 antigenemia (>1,000 pg/mL) was detected. Ten days after the splenectomy was performed, an ELISA and western blot were positive for HIV-1, and the antigenemia began to decrease.

The present case illustrates a severe clinical picture of primary HIV-1 infection that was manifested by thrombocytopenia and spontaneous rupture of the spleen. To our knowledge, only one other case of HIV seropositivity revealed by spontaneous

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rupture of the spleen has been reported, but it was not possible to determine the stage of HIV infection in that case [1]. There are many hypotheses regarding the mechanism of the splenic rupture, including lymphoid infiltration of trabecular veins, as has been described in cases of Epstein-Barr virus infection, as well as endothelial cell dysfunction and acute vasculitis [2]. In our patient's case, lymphoid infiltration of trabecular veins was the main feature of his condition, and no acute vasculitis was noted. His thrombocytopenia likely played an important but insufficient role in the development of the hematoma.

We conclude that in all cases of spontaneous rupture of the spleen in which no other etiology can be determined, it seems appropriate to screen for acute HIV infection by means of ELISA and measurement of p24 antigenemia.

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Recurrent Iritis After Intravenous Administration of Cidofovir

Although iritis is a relatively common side effect with intravitreal injections of cidofovir [1, 2], this adverse drug reaction rarely occurs with administration of the iv form, and its occurrence has not yet been clearly linked to administration of this drug [3]. We describe a case of recurrent unilateral iritis following iv administration of cidofovir (Vistide, Gilead Sciences, Foster City, CA) to a patient with AIDS and cytomegalovirus (CMV) retinitis.

A 41-year-old HIV-infected homosexual male (CD4 cell count, 15/mm^3) with a history of cryptococcal meningitis in 1993 and unilateral CMV retinitis (right eye) presented to the Ochsner Clinic in New Orleans on 26 November 1996 with a 4-day history of right red eye and photophobia. He denied ocular pain, visual blurring, or any constitutional symptoms. His temperature was 37.0°C, his blood pressure was 110/70 mm Hg, and his heart rate was 85. His right conjunctiva was hyperemic and inflamed, but the rest of the physical examination was unremarkable. Except for a WBC count of 3.0×10^3, his laboratory values were within normal limits.

CMV retinitis of the right eye had been diagnosed in January 1995. At that time, therapy with iv ganciclovir was started; however, the patient developed a skin rash and his therapy was switched to foscarnet. Although his CMV retinitis remained stable for 22 months, therapy was changed to iv cidofovir because of its convenient administration (every other week rather than daily).

On 23 October 1996, he received the first dose of cidofovir (5 mg/kg or 340 mg) along with iv saline and 4 g of oral probenecid. The patient did not report any complications or side effects. On 6 November 1996, he received the second dose of cidofovir and probenecid. Two days later he called to report the onset of a right eye inflammation and photophobia. He refused to be examined, but 6 days later he called again to report that the symptoms had spontaneously disappeared. On 20 November 1996 he received the third dose of cidofovir and probenecid, and 2 days later he noticed the recurrence of right eye inflammation and photophobia. This time he decided to come to the clinic.

The patient's other medications were ritonavir, zalcitabine, lamivudine, fluconazole, and aerosolized pentamidine (given monthly). Ophthalmologic examination revealed a visual acuity of 20/20 normal vision in both eyes. The right eye had 1+ bulbar conjunctival injection and 1–2+ cell and flare in the anterior chamber. The left cornea was clear, but inferior keratic precipitates were seen on the right inferior corneal endothelium. His CMV retinitis was quiescent. The vitreous had no cells or posterior vitreous detachment. Numerous examinations performed before cidofovir therapy was begun showed no signs of anterior uveitis.

The patient received treatment with topical 1% prednisolone acetate eight times daily until the inflammation resolved. As of 18 May 1997, he had had three consecutive episodes of iritis after the administration of cidofovir without visual loss.

Cidofovir is an acyclic cytosine nucleoside phosphonate analog that is highly active against CMV. Its iv formulation was approved by the Food and Drug Administration in June 1996 for the treatment of CMV retinitis in patients with AIDS. Significant adverse reactions to cidofovir include elevation of the creatinine level, proteinuria, neutropenia, and metabolic acidosis. In clinical trials, a decrease in intraocular pressure (hypotony) was the most common ocular adverse reaction observed, and this reaction occurred in 12% of the patients. Other ocular adverse effects encountered and listed in the prescribing information for cidofovir as ''regardless of causal relationship'' include amblyopia, conjunctivitis, iritis, retinal detachment, uveitis, and abnormal vision.

In contrast, the intravitreal form of cidofovir was shown to cause a variety of ocular complications, including iritis, uveitis, vitreitis, and hypotony [1, 2]. In one study with low-dose (20-mg) intravitreal cidofovir for CMV retinitis, iritis occurred in 14% of the patients who received prophylaxis with oral probenecid and in 41% of the patients who did not receive probenecid [1]. The presumed role of probenecid is to lower the uptake of cidofovir into the ciliary body. This appears to have an effect on decreasing the incidence of ocular hypotony and iritis [1]. Probenecid helps prevent uptake of cidofovir into epithelial tissues by competitive inhibition. Damage to this ciliary body epithelium results in hypotony as well as in inflammation secondary to tissue damage or irritation.

According to a report received by the manufacturer (Gilead Sciences; J. Buchanan, personal communication), 10 cases of iritis had been associated with the administration of iv cidofovir as of

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December 1996. None of those cases has been published in the literature; the clinical characteristics of the patients, the possible interaction of cidofovir with other medications, and the patient outcomes are unknown. Clinicians who treat CMV retinitis with cidofovir should be aware of this rare complication.

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Rapid Emergence of Resistance to Cefepime During Treatment

Cefepime is a newly licensed extended-spectrum cephalosporin that has been shown to possess activity in vitro against ceftazidime-resistant strains of *Enterobacter* and to effectively treat infections caused by such organisms in vivo [1, 2]. Cefepime also appears less likely than other cephalosporins to select for resistant strains of *Enterobacter* during therapy in animal models [3, 4]. Despite these potential advantages, we report a case of clinical failure of cefepime because of the rapid emergence of resistance during treatment of a liver abscess due to *Enterobacter aerogenes* in a liver transplant patient.

See editorial response by Medeiros on pages 341–2.

A 47-year-old man underwent orthotopic liver transplantation on 22 June 1996 because of cirrhosis due to hepatitis C. Postoperative complications included hepatic artery thrombosis and hepatic abscesses requiring percutaneous drainage. He was admitted to the hospital on 16 September 1996 with fever and abdominal pain, and cultures of blood and hepatic abscess fluid yielded *E. aerogenes* that was susceptible to ceftazidime, ciprofloxacin, and cefepime, as determined by disk diffusion (table 1). Radiographic studies revealed a hepatopleural fistula, and the patient underwent thoracoscopy, tube thoracostomy, and additional percutaneous drainage of the hepatic abscesses. On the basis of the initial susceptibilities of the blood and hepatic abscess isolates, treatment with ciprofloxacin was begun.

After an initial clinical response, repeated blood cultures performed on 20 September and 22 September again yielded *E. aerogenes* with susceptibilities that were identical to those of the previous isolates, and ceftazidime was added to the patient's antibacterial regimen (table 1). Despite treatment with ceftazidime and ciprofloxacin and additional percutaneous drainage of the hepatic abscesses, the patient became febrile, and blood cultures performed on 29 September and 3 October again yielded *E. aerogenes;* however, the isolate was now resistant to ceftazidime and a number of other β-lactams as well as ciprofloxacin (table 1). The *E. aerogenes* blood isolates recovered on 29 September and 3 October were susceptible to cefepime, as determined by disk diffusion (zone size, 27 mm), use of the Etest (MIC, 0.5 μg/mL), and broth dilution (MIC, 0.5 μg/mL).

On 3 October, cefepime (2 g iv q12h) was substituted for ceftazidime. One week after treatment with cefepime was begun, the patient again became febrile, and blood cultures performed on 10 October and 11 October yielded *E. aerogenes;* however, the isolate was now resistant to cefepime when disk diffusion (zone size, 12 mm), the Etest (MIC, 48 μg/mL), and broth dilution (MIC, 32 μg/mL) were performed (table 1). Imipenem was substituted for cefepime on 13 October, and the patient responded with resolution of fever and a reduction in the size of the hepatic abscesses over the next several weeks; he had no further episodes of enterobacter bacteremia. He eventually underwent repeated liver transplantation on 17 November and was discharged on 27 November.

Table 1. Antimicrobial susceptibilities of *Enterobacter aerogenes* isolates, as determined by disk diffusion.

Date of blood culture	Antimicrobial tested										
	Gm	Atm	Cpfx	Pip	Tic/Clv	Cfaz	Ctan	Czid	Ctri	Cfep	Imi
16 September	S	S	S	S	S	R	S	S	S	S	S
20 September	S	S	S	S	S	R	S	S	S	S	S
29 September	S	R	R	R	R	R	R	R	R	S	S
3 October	S	R	R	R	R	R	R	R	R	S	S
10 October	S	R	R	R	R	R	R	R	R	R	S
11 October	S	R	R	R	R	R	R	R	R	R	S

NOTE. All disk diffusion susceptibility tests were performed as described in the National Committee for Clinical Laboratory Standards guidelines. Each cefepime disk susceptibility result was confirmed by broth dilution and Etest methods (data not shown). Atm = aztreonam; Cfaz = cefazolin; Cfep = cefepime; Cpfx = ciprofloxacin; Ctan = cefotetan; Ctri = ceftriaxone; Czid = ceftazidime; Gm = gentamicin; Imi = imipenem; Pip = piperacillin; R = resistant; S = susceptible; Tic/Clv = ticarcillin/clavulanate.

Sanders et al. [2] reported the efficacy of cefepime for treating infections due to multidrug-resistant *Enterobacter* species and found no evidence of the emergence of resistance during therapy in the 16 patients studied. In contrast, resistance to cefepime developed in our patient after only 7 days of therapy. Strain typing by pulsed-field gel electrophoresis documented emergence of resistance in the original *E. aerogenes* isolate rather than selection of a different resistant strain (data not shown).

Although cefepime has been reported as efficacious for treating resistant gram-negative infections and there have been no

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previous reports of the emergence of resistance during therapy, we documented the failure of cefepime therapy for a liver abscess due to *E. aerogeues;* this failure resulted from rapid emergence of resistance to cefepime. Studies to identify the specific mechanism(s) of resistance in this isolate are currently in progress and may help identify potential mechanisms of resistance to other extended-spectrum cephalosporins.

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Editorial Response: Relapsing Infection Due to *Enterobacter* Species: Lessons of Heterogeneity

One of the most curious and puzzling aspects of infections due to *Enterobacter* species is the predilection of these organisms to develop antimicrobial resistance during therapy, especially to the third-generation cephalosporins. Resistance to oxyimino-cephalosporins has emerged during therapy in 19% of patients treated for enterobacter bacteremia in academic medical centers in the United States [1]. Rates are highest for patients with high-density infections such as deep-seated abscesses and pneumonia; however, rates of emergence of antibiotic resistance during therapy are much lower among patients with urinary tract infections, where β-lactams reach very high concentrations [2].

See article by Limaye et al. on pages 339–40.

The basis for this phenomenon is rooted in the complex mechanism by which *Enterobacter* species resist β-lactam antibiotics. These organisms typically produce small amounts of a chromosomally encoded class C β-lactamase that has a high affinity for third-generation cephalosporins but a low maximum hydrolysis rate. As a consequence, the β-lactamase mediates resistance to these antibiotics only when it is produced in large quantities. Several genes regulate its production, but mutation of only one of these genes, *amp*D, results in constitutive hyperproduction of the enzyme [3]. For reasons that remain a mystery, these mutations occur spontaneously at rates as high as 10^{-4} to 10^{-6} [4], resulting in a heterogeneous population of bacterial cells with differing levels of β-lactam resistance.

Exposure to a third-generation cephalosporin such as ceftazidime selects for hyperproducer mutants. These mutants are typically resistant to cefotaxime, ceftazidime, aztreonam, ceftriaxone, piperacillin, and ticarcillin/clavulanate. Moreover, many clinical isolates of *Enterobacter* species also produce a plasmid-mediated β-lactamase—most commonly TEM-1—that may contribute further to resistance [5, 6].

The degree of resistance also depends on the rate at which the β-lactam penetrates into the bacterial cell. Slow penetration into the periplasmic space, where the β-lactamase is sequestered, increases the enzyme's efficiency in hydrolyzing the β-lactam antibiotic. Porins, proteins that are embedded in the outer membrane of gram-negative bacilli, act as channels through which β-lactams enter the cell. Different strains of *Enterobacter cloacae* vary greatly in the amounts of porins produced [7], perhaps because a single *Enterobacter* gene, *rom*A, regulates the expression of porins [8]. This variation further contributes to the heterogeneity of resistance in *Enterobacter* species. Exposure to imipenem or meropenem, β-lactams that are hydrolyzed very slowly by the class C *Enterobacter* β-lactamase [4], selects for resistant porin-deficient variants in vitro; however, this selection remains rare in vivo [9–13].

Cefepime, a new oxyimino-cephalosporin with a positively charged quaternary ammonium at carbon 3 of the dihydrothiazone ring, promised to circumvent both of these mechanisms. This drug has a lower affinity for the chromosomal β-lactamase of *Enterobacter* species, and, as a zwitterion, cefepime has much higher permeability across the outer membrane than other cephalosporins [14]. The MICs of cefepime for *Enterobacter* rose from 0.06 μg/mL to 2.0-4.0 μg/mL upon selection of hyperproducer mutants by ceftazidime, but these MICs were still within the range of susceptibility [15]. Cefepime was used to successfully treat 16 patients infected with ceftazidime-resistant isolates of *Enterobacter* species, and relapses due to cefepime-resistant strains did not occur [16]. It appears that cefepime could be used to treat enterobacter infections more reliably than other oxyimino-cephalosporins.

The case reported in this issue of *Clinical Infectious Diseases* dramatically underscores several caveats to this conclusion. The patient, a liver transplant recipient with recurrent liver abscesses, developed bacteremia due to an isolate of *Enterobacter aerogenes* that was susceptible to ceftazidime, cefepime, and ciprofloxacin. Despite percutaneous drainage and therapy with ciprofloxacin, the patient's bacteremia recurred with the same strain 4 days later, probably because drainage of the abscesses was inadequate. At that point, ceftazidime was added to the regimen. Within 7 days, blood cultures yielded a typical ceftazidime-resistant hyperproducer mutant that was resistant to most β-lactams but susceptible to cefepime (MIC, 0.5 μg/mL; zone of inhibition, 27 mm). A decision was made to administer cefepime.

Are ceftazidime-resistant enterobacters truly susceptible to cefepime? Johnson et al. tested 56 ceftazidime-resistant isolates of *E. cloacae* by means of agar dilution with inocula of 10^4 cfu/mL and 10^7 cfu/mL [15]. Although 96% of the isolates tested susceptible to cefepime with use of the standard 10^4 inoculum, all were resistant when the larger inoculum was used. Similarly, Pechere et al. [17] found that ceftazidime-resistant strains were susceptible by standard agar dilution. However, a gradient-plate method showed that the ceftazidime-resistant strains contained a subpopulation highly resistant to cefepime (MICs, 128–256 μg/mL) [11].

Would cefepime nevertheless be effective in vivo against ceftazidime-resistant strains that contain a minority resistant subpopulation? In a peritonitis model, cefepime cured 17 of 18 mice infected with ceftazidime-susceptible *E. cloacae* but only 7 (39%) of 18 mice infected with a ceftazidime-resistant strain [18]. The implication of these studies is that selection of variants resistant to cefepime may be a two-step process; the first step is selection of a hyperproducer mutant, something that other oxyimino-cephalosporins are quite able to do, and the

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second step is selection of a permeability mutant that confers a higher level of resistance. In this regard, Fung-Tomc [18] found that 80% of the resistant variants selected by stepwise passage in broth containing cefepime had diminished levels of a porin protein. In contrast, only 10% of the resistant variants selected by ceftazidime had decreased porins. It is of interest that resistance to cefepime developed more rapidly in those strains that produced both a chromosomal β-lactamase and a TEM-type β-lactamase.

The outcome for the patient of Limaye et al. appears to mirror these results. One week after he received cefepime, his fever recurred, and blood cultures yielded. *E. aerogenes* resistant to ceftazidime, ciprofloxacin, and cefepime (MIC, 32 μg/mL; zone diameter, 12 mm). His condition improved after treatment with imipenem. He had a high-density infection (poorly draining liver abscesses) in a location where achieving very high levels of antibiotic was unlikely. In addition, selection of a hyperproducer mutant had already occurred at the time he first received cefepime. As predicted by the animal model, the patient was thus at high risk of treatment failure with cefepime [17]. It seems likely that under these conditions, cefepime selected for a porin-deficient mutant. Hopefully, further studies of these strains will clarify this possibility.

This case provides several lessons. Cefepime should probably not be used in patients who have high-density infections (e.g., pneumonia or deep abscesses) caused by ceftazidime-resistant strains of *Enterobacter* species. If cefepime is used to treat infections due to ceftazidime-susceptible strains, the monitoring of therapy with quantitative susceptibility testing (i.e., determining zone sizes or MIC values) is crucial. The emergence of the hyperproducer mutant phenotype (with resistance to cefotaxime or ceftazidime and a higher MIC or smaller zone of inhibition to cefepime albeit within the range of susceptibility) should trigger a reexamination of therapeutic options.

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