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Case Report

Edema and Tetraparesis in a Miniature Pig after Allogeneic Hematopoietic Cell Transplantation

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A 3-mo-old, 12-kg, intact, miniature pig presented with severe neurologic signs on day 8 after hematopoietic cell transplantation. This pig had received an immunosuppressive regimen before transplantation that included an antiCD3 immunotoxin for T-cell depletion, 100 cGy of total-body irradiation, and cyclosporine for 45 d. The pig began exhibiting erythematous lesions on posttransplantation day 7. He also demonstrated increased conscious proprioceptive deficits and recumbency but normal mentation. Neurologic signs worsened over several days; the pig became lethargic but remained afebrile. Conjunctival swelling developed on posttransplantation day 9, which subsequently spread to the animal's head, ears and hocks by day 10. Analgesics were given for pain, and cyclosporine levels were decreased. Despite the measures taken, neurologic signs progressed. Given the worsening subcutaneous edema and neurologic status, *Escherichia coli* infection was suspected, and treatment with a third-generation cephalosporin was instituted. The clinical signs resolved within 12 h after the start of antibiotics. 'Shiga-like' toxin from *E. coli* can cause peracute toxemia and induce ataxia, paralysis, and recumbency. Other common and pathognomonic findings include periocular edema and variable edema in other subcutaneous regions. A fecal sample demonstrated an overgrowth of gram-negative, lactose-fermenting colonies. On the basis of the clinical presentation, exclusion of other potential conditions compatible with edema and neurologic diseases, physical exam findings, microbiology and the resolution of signs after therapy, the pig was diagnosed with edema disease.

Abbreviations: HCT, hematopoietic cell transplantation; PTD, posttransplantation day.

Hematopoietic cell transplantation (HCT) is a common therapy for many hematologic malignancies including leukemia and lymphoma.[5] Patients undergoing HCT are maintained under immunosuppression and are at increased risk of acquiring viral, bacterial, and fungal infections.

In our laboratory we use miniature pigs of defined major histocompatibility complex as a large animal model for the study of HCT.[22,23,27,28] The pig is an excellent and well-accepted large animal model in biomedical research[15,19] Pigs that undergo HCT develop transplantation-related complications that are very similar to what is reported in the human clinic, such as posttransplantation lymphoproliferative disease[7,17] and thrombotic microangiopathy.[18] However, swine pathogens can often be overlooked in lieu of typical transplantation-related complications. Vigilance for veterinary pathogens is important, and their identification is crucial. Failure to identify these pathogens may have a detrimental impact on experimental outcomes. Here we describe a case of edema disease in an immunosuppressed miniature pig after HCT. Edema disease due to *Escherichia coli* is a common condition in swine[11,13,35] and has been used as a model in microbiologic studies.[6] The symptomatology resembled and overlapped with known and relatively common posttransplantation complications. For these reasons, diagnosis was particularly challenging in this case. To our knowledge, this is the first report of edema disease in an animal undergoing hematopoietic cell transplantation.

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Materials and Methods

Animals. The pig presented is an MGH major-histocompatibility-complex–defined miniature swine (*Sus scrofa domestica*) from a closed SPF herd (free of pseudorabies, porcine reproductive and respiratory syndrome, and transmissible gastroenteritis viruses and *Brucellosis* spp.) at an AAALAC-accredited institution. The immunogenetic characteristics of this herd have been previously reported.[19,26] Swine are vaccinated for *Mycoplasma hyopneumoniae*, *Hemophilus parasuis*, *Streptococcus suis*, *Pasturella multocida*, *Bordatella bronchiseptica*, *Erysipelothrix rhusiopathiae*, and porcine circovirus strains 1 and 2. In addition, the pigs are dewormed with ivermectin prior to shipment. Approximately 50 miniature swine of varying ages are housed in our large animal facility. Pigs undergoing HCT are housed in conventional steel cages with HEPA filters. All transplants were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care.

Conditioning. After an acclimation period of approximately 14 d, a miniature pig underwent bone marrow and thymic biopsies before transplantation according to laboratory protocol, as well as surgical placement of intravenous catheter and gastric tubes. In

brief, the external jugular vein was approached by ventral midline neck incision, and an indwelling central catheter was placed. The catheter was tunneled 40 to 60 cm through the subcutaneous layers and exited over the scapular region. The thymus was approached similarly through a midline ventral neck incision, and a sample of cervical thymus was taken. Gastric tube placement was approached via abdominal laparotomy. The linea alba was identified and the abdominal cavity entered. A small incision was made, the gastric tube was inserted into the stomach, and 2 purse-string sutures were placed to secure the tube. The gastric tube was tunneled through the left side of the body wall, and the stomach was fixed to the abdominal wall.

The pig began a 4-d course of porcine antiCD3 recombinant immunotoxin (50 µg/kg twice daily), 4 d prior to transplantation.[36] At 2 d before transplantation, the pig received 100 cGy of total body irradiation, and the standard calcineurin inhibitor cyclosporine A was provided twice daily through the G tube, beginning the day before HCT. Trough serum levels for cyclosporine A were measured daily, and doses were adjusted appropriately to maintain trough levels of 400 to 800 ng/mL for 30 d, after which levels were tapered until day 45. To prevent gastrointestinal fungal infections, the pig also received 100,000 U nystatin twice daily. During the neutropenic period after conditioning, the pig received a 10-d course of prophylactic enrofloxacin according to our standard protocol.

Transplant. Beginning on PTD 0, the pig received an HCT comprising 7.5×10^9 total peripheral blood mononuclear cells from a haploidentical donor; cells were delivered intravenously over 2 d. Cells were mobilized for collection by giving the donor pig a combination of porcine IL3 and pig stem cell factor for 5 d, followed by leukapheresis until sufficient cells were collected.

Case Report

A 3-mo-old, 12-kg, intact male miniature pig presented with coalescing erythematous skin lesions and subcutaneous swelling that started on day 7 after haploidentical HCT. The erythematous lesions were present primarily on the ears, neck, and base of the tail. In addition, edema was noted on the abdomen and over the lateral aspects of both hindlegs (Figure 1 B). On the evening of day 8, progressive ataxia and paraparesis of the rear limbs was observed: the pig was dragging its feet and appeared to be weak. In addition, the pig appeared disoriented and began to 'head press.' Although pain responses were not elicited on palpation of the abdomen, hocks, elbows, neck, or back, oral tramadol was started to treat for other, nonspecific pain. In addition, conscious proprioceptive deficits (particularly in the hindlegs) were present, and tarsal flexing and scuffing of the hooves were observed. Daily trough levels of cyclosporine were evaluated according to protocol. Cyclosporine toxicity was considered in our differential diagnosis, because high levels of this drug have been shown to cause tremors and paresthesia as well as liver and kidney toxicity in humans.[14] However, the pig's trough levels were 350 ng/mL at the onset of neurologic signs (posttransplantation day [PTD] 8; Figure 2 B).

On PTD 9, the edema had worsened and affected the eyelids and conjunctiva (Figure 1 A). Aerobic, anaerobic, and fungal blood cultures were obtained, all of which were negative. In addition, daily analysis of the pig's serum blood chemistries showed normal total protein and albumin, liver (AST and ALT; Figure 2 D), and kidney (creatinine and BUN; Figure 2 C) parameters, all

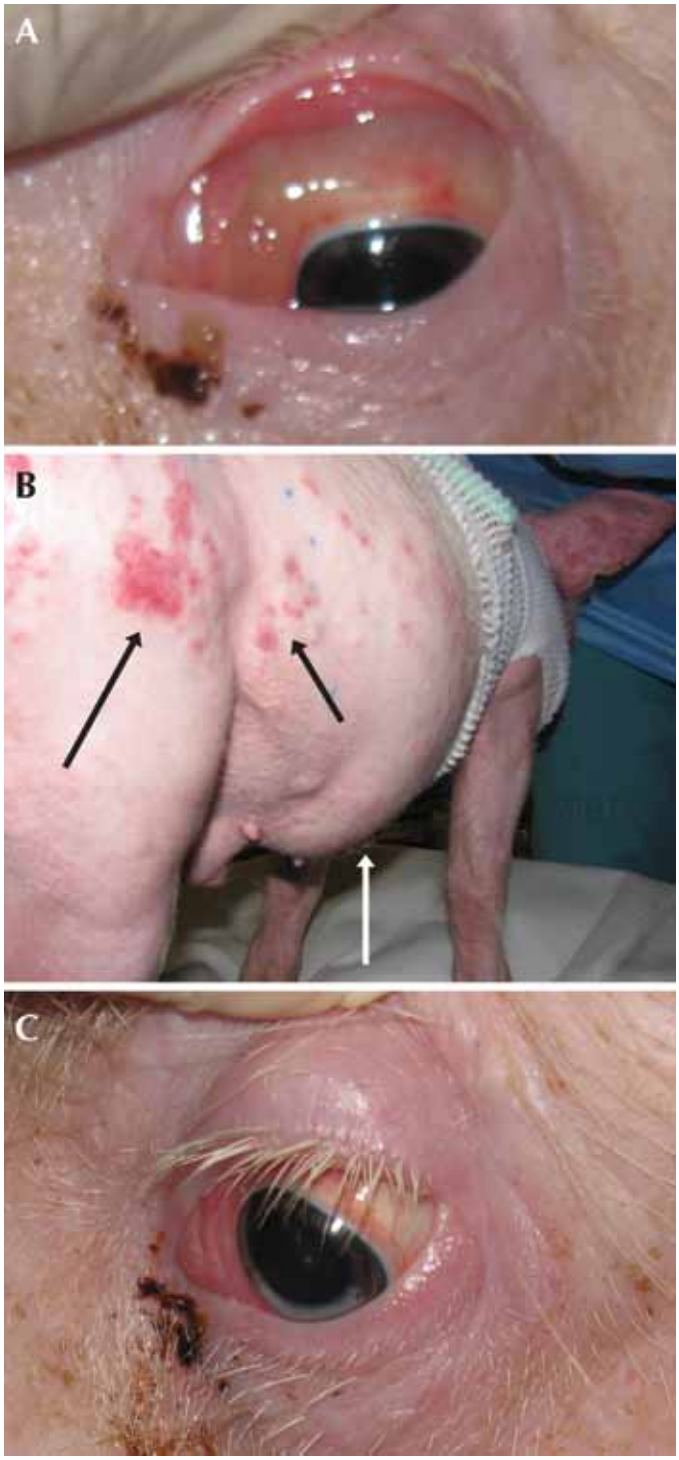

Figure 1. (A) The left conjunctiva on day 9 after transplantation: clear edema was present in the palpebral and conjunctival surfaces. (B) Edema spread to the abdomen and hocks by posttransplantation day 10 (solid arrow). Erythematous skin lesions affected the right flank (dashed arrow). (C) The left eye at 48 h after start of appropriate antibiotic treatment. Edema is reduced greatly but not fully resolved; eye assessment was performed without sedation.

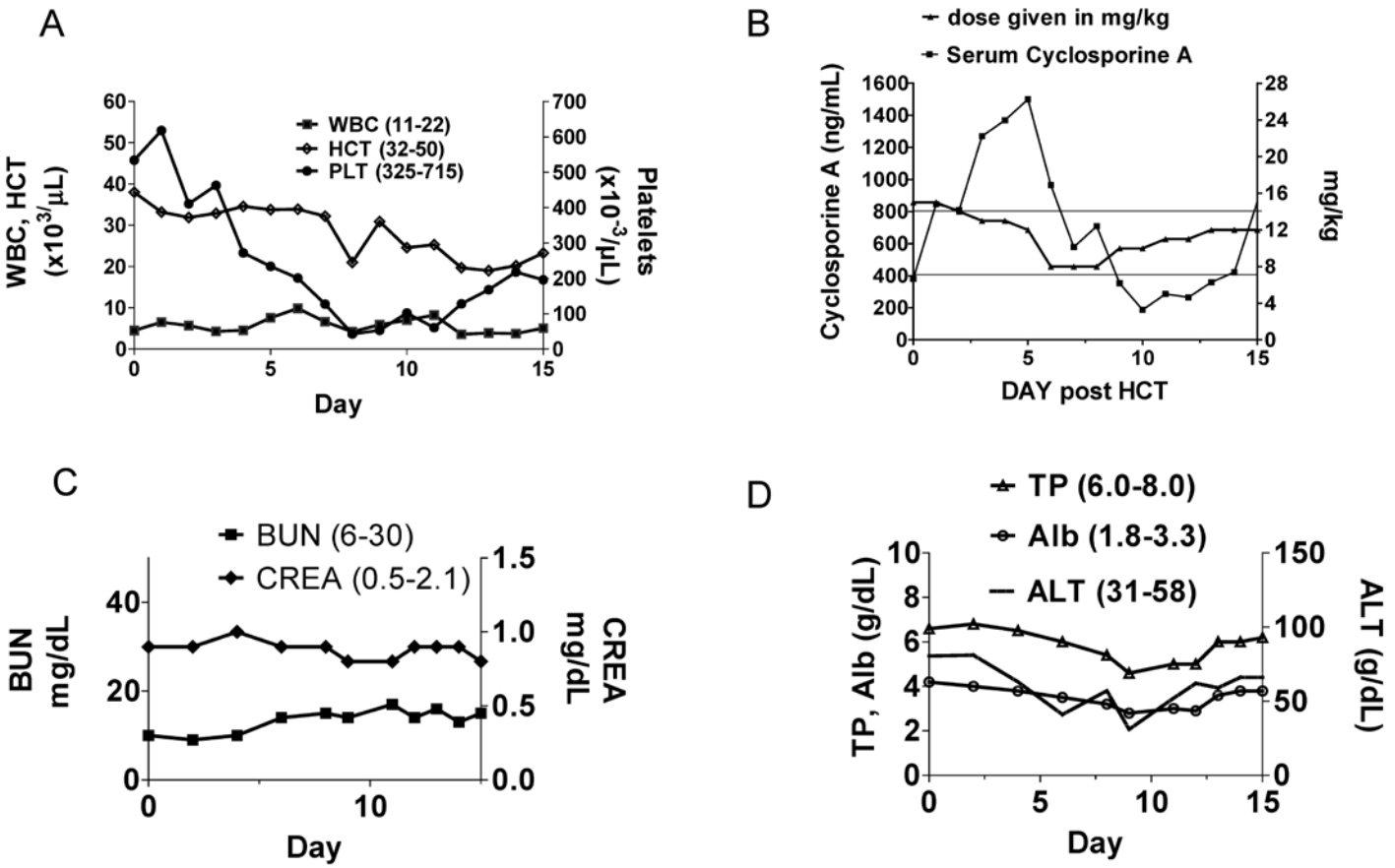

Figure 2. (A) WBC count, hematocrit (HCT), and platelets (PLT) during the first 15 d after transplantion. (B) Serum levels and dose (mg/kg) of cyclosporine delivered via the gastric tube. Horizontal lines represent the therapeutic cyclosporine target levels of 400 to 800 ng/mL. (C) BUN and creatinine (CREA) levels throughout the course of illness. (D) ALT and liver function parameters (total protein [TP] and albumin [Alb] levels).

of which can be affected in cases of drug toxicity or graft-versus-host disease.[2,16] The pig was leukopenic and thrombocytopenic, with a nadir of 43 K/µL platelets on PTD 8 (Figure 2 A). Although the skin lesions may have been consistent with thrombocytopenia or skin graft-versus-host disease, platelet levels exceeded those typically seen to induce petechiation (approximately 20,000/µL), and the skin lesions did not follow the pattern and distribution associated with classic graft-versus-host disease.[12]

The edema had spread to the pig's head, ears, and hocks by PTD 10. In addition, his neurologic signs had worsened to tetraparesis, and was unable to stand, had a lack of motor function, and still appeared to have superficial pain. The pig had difficulty raising its body with its front legs, had conscious proprioceptive deficits in all 4 limbs, and typically rested over both carpus. The pig was lethargic and inappetant but remained afebrile. The prophylactic antibiotic regimen consisting of enrofloxacin and metronidazole was discontinued on PTD 10 according to protocol. This decision was reinforced by the facts that the pig was afebrile; his WBC count was recovering (with greater than 1000 neutrophils/µL; Figure 2 A); and both fluoroquinolones and metronidazole potentially could be responsible for the neurologic signs observed. Implementation of these measures did not improve the pig's condition and, in fact, he continued to deteriorate.

On PTD 10 a fecal culture was also taken for analysis. The edema continued to be of concern, and its localized presentation in the eyelid was crucial for the successful treatment of this animal. Based on these findings and our suspicion of Edema disease, the animal was started on a broad-spectrum, third-generation cephalosporin (ceftriaxone; 20 mg/kg twice daily for 14 d). Although we lacked diagnostic positive blood or fecal cultures, many gram-negative bacteria (particularly *E. coli* strains) are susceptible to this class of antibiotics.[21]

By 18 h after commencement of antibiotic therapy (that is, PTD 11 to 12), the pig had recovered considerably from his neurologic signs. He was ambulatory, although still weak and exhibiting some conscious proprioceptive deficits. The edema of the conjunctiva had decreased by 90% (Figure 1 C), and both the edema and neurologic signs were nearly imperceptible by 48 to 72 h after the initiation of antibiotic therapy (PTD 12 to 13). The fecal sample taken on PTD 10 grew mucoidal gram-negative, lactose-fermenting cocci on McConkey plates, compatible with *E. coli*. On the basis of the clinical presentation (especially conjunctival edema), neurologic signs, culture results, and prompt resolution after initiation of ceftriaxone, edema disease was diagnosed after exclusion of other transplant-related complications.

Discussion

Edema disease is an enteric illness caused by α-hemolytic *E. coli* that colonize the small intestine and produce a Shiga-like toxin, Stx2e.[20] This disease is somewhat similar to human diseases

caused by enterohemorrhagic strains of *E. coli*, which produce closely related, but not identical, Shiga toxins. These human strains colonize the intestine and, like those in swine, have several serotypes. In swine, the *E. coli* serotypes most commonly involved in edema disease are O138, O139, and O141.[10] The serotype O139 has been found globally to produce the fimbrial variant F18ab and induce edema disease. Susceptibility is determined by the presence (or absence) of receptors for these fimbriae on the small intestinal epithelial cells.[10] Genetic resistance to pathogenic *E. coli* can be bred into a herd, because the F18 gene is inherited as a dominant trait and found on a single locus.[11]

The diagnosis of acute edema disease is based on the sudden appearance of neurologic signs. Edema disease usually affects postweaning piglets, which present with ataxia and a staggering gait. Usually edema is severe under the eyelid and often involves other areas of the body (typically ventral areas); the subcutaneous edema in the palpebrae and over the frontal bones is a cardinal sign. Skin reddening is present, and affected pigs typically are afebrile, as was our animal.[31] The prognosis of pigs with advanced-stage disease and severe subcutaneous edema, respiratory distress, or the inability to rise is grave. It is often difficult to treat pigs for edema disease when the condition is in this advanced stage because the toxins have already bound to their receptors.[31] Third-generation cephalosporins and fluoroquinolones are broadly effective against gram-negative bacteria, but some authors[31] have questioned the use of antibiotics during the acute phase of the disease,[32] because they potentially can induce dying bacteria to release more toxin. Therefore, antibacterial agents in these cases are ineffective. However, in our pig, attention to his clinical appearance and swift treatment (before he became recumbent and moribund) by using an antimicrobial drug susceptible to *E. coli* (ceftriaxone) effectively eliminated the infection within 2 d. Although enrofloxacin has been effective against edema disease in pigs,[32] it appeared to be ineffective in our pig. Because our transplantation protocol uses enrofloxacin as the standard prophylactic antibacterial medication, a fluoroquinolone-resistant strain of bacteria may have been responsible for the disease in this case. We were unable to assess the antibiotic sensitivities of the lactose-fermenting mucoidal bacterial colonies grown from the fecal sample. Of note, the use of enteric nystatin and cyclosporine may have affected the composition of the bacterial flora.

The necropsy findings from a pig with edema disease can be variable. Often edema is present in the mesentery, stomach, and mesocolon.[11] In addition, the lungs may be affected, and a characteristic patchy, sublobular congestion may often be the only observable lesion. The skin lesions and edema are caused by the swelling of the vascular endothelium as well as deposition of microthrombi, leading to the characteristic erythema of this disease. Most striking are the microscopic lesions indicating a degenerative angiopathy affecting small arteries and arterioles.[4] The degree of bacterial colonization in the gastrointestinal tract determines whether disease occurs. The F18 receptor in the brush border serves as a target for *E. coli* strains responsible for edema disease.[34] Changes in feed may cause enhanced colonization of F18-positive *E.coli*.[1] Bacterial Stx2e toxin is absorbed into the circulation and bound to globotetraossyl ceramide on RBC. In addition, toxin can be found in the endothelial cells and in the microvillous membranes of enterocytes.[35] Therefore, the most consistent injury observed is a degenerative angiopathy of small arteries and arterioles. The edema in the tissues is low in protein and attributed to increased vascular permeability.[13] Histologically, patchy layers of adherent bacteria throughout the small intestine can be observed. However, despite bacteria-positive samples obtained early during the course of infection, bacterial colonization often disappears by the time the animals become moribund. Often, the only definitive sign in an animal that has survived an infection is a focal encephalomalacia in the brain stem, a lesion that is believed to be due vascular injury leading to edema and ischemia. These lesions are thought to be responsible for the neurologic signs.[11]

Because PCR tests[3] demonstrating the presence of a Shiga toxin are necessary for definitive diagnosis but were not performed in our case, we had to rule out alternative pathogens known to cause edema, neurologic signs, and erythema, including *Escherichia coli* (known to cause Edema disease),[11] *Clostridium septicum*,[24] *Haemophilus parasuis*,[25] porcine circovirus,[29] and (less likely) paramyxovirus.[30] Of these, paramyxovirus infection can cause severe neurologic signs but would not have improved with antibiotics. *Haemophilus parasuis* is a bacterial pathogen that mainly causes fevers, muscle tremors, recumbency, and edema, although not frequently observed to affect the eyelid.[25] In comparison, our pig not only remained afebrile throughout his clinical course but also had been vaccinated against *Haemophilus* and porcine circoviruses 1 and 2. Common gram-negative and lactose-fermenting bacterial pathogens that can be found in pigs include *Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter* spp., and *E. coli*. Of all these pathogens, only enterotoxigenic *E. coli* characteristically induces the presentation observed. Our pig was treated relatively early and never progressed to full recumbency or respiratory distress.

The clinical manifestations in our case enabled us to rule out several important transplantation-related conditions. When working with immunocompromised patients undergoing transplantation and that require close monitoring of their immune status, signs of sickness represent a diagnostic and treatment challenge. Similar to the current case, bacterial infections are not uncommon in immunocompromised persons undergoing HCT, and *E. coli* continues to be one of the multiple pathogens that garner clinicians' attention.[8,33] Addition or discontinuation of any of the drugs that an animal is receiving potentially could have a detrimental effect, including graft-versus-host disease,[9,10] rejection of the donor graft, and worsening of any potential infection.[5] When the signs of typical peritransplantation complications and alternative etiologies overlap, as occurred in our case, clinical judgment and experience play an important role in the treatment approaches. Our findings are important for others working with large animals undergoing immunosuppression. Species-specific bacterial pathogens need to be considered in animal transplantation studies when familiar peritransplantation complications fail to explain the clinical picture. In this report, we demonstrate how frequent monitoring and reassessment of our pig was crucial for the resolution of the case. In light of all differential diagnoses, the symptomatology (especially neurologic signs with the concomitant cardinal presentation of conjunctival edema) and the quick response to an antibiotic to which *E. coli* is sensitive, edema disease was diagnosed. In summary, and to our knowledge, this case represents the first report of edema disease in an immunocompromised animal undergoing HCT.

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