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CASE REPORT

Vaccine strain *Listeria monocytogenes* bacteremia occurring 31 months after immunization

Elias Fares[1,5] · Cindy B. McCloskey[2] · Andres Gutierrez[3] · Michael Princiotta[3] · Linda J. Salinas[1] · Douglas A. Drevets[1,4]

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Abstract

Background *Listeria monocytogenes* is a food-borne, facultative intracellular bacterium that causes severe diseases such as sepsis and meningoencephalitis in immunocompromised hosts. Because it stimulates robust T-lymphocyte-mediated responses, attenuated *L. monocytogenes* are candidate vaccine vectors for tumor immunotherapy.

Case We report a case of bacteremia caused by vaccine strain *L. monocytogenes* (*Axalimogene filolisbac*) occurring 31 months after immunization against human papilloma virus (HPV) associated cervical cancer.

Conclusion Receipt of a *L. monocytogenes*-based vaccine is a novel risk factor for delayed *L. monocytogenes* bacteremia.

Keywords Immunotherapy · Live vaccine · Recurrent listeriosis. *Listeria* vaccine

Introduction

L. monocytogenes is a facultative intracellular bacterium that causes severe diseases, in particular sepsis and a variety of central nervous system infections [1]. The average annual incidence of invasive *L. monocytogenes* infections in the United States is calculated at 0.29 cases per 100,000, but is significantly higher in certain at risk groups such as adults aged ≥ 65 years, pregnant women, patients with solid organ and hematologic malignancies, and individuals who receive immunosuppressive therapy [2, 3]. *L. monocytogenes* mutants with attenuated virulence have been developed as vaccine vectors for tumor immunotherapy because listerial antigens are presented by MHC class I and class II molecules during infection and stimulate strong CD8 and CD4 T-lymphocyte responses [4, 5]. Despite attenuation however, live vaccines can possibly cause infections of unintended severity in recipients. We report a case of bacteremia caused by an attenuated strain of *L. monocytogenes*, known as *Axalimogene filolisbac* (AXAL, ADXS11-001, *Lm*-LLO-E7) [6], occurring in a subject 31 months after immunization against human papilloma virus (HPV)-associated cervical cancer.

Case report

A 56-year-old female with progressive cervical cancer was enrolled in a Gynecologic Oncology Group immunotherapy trial using the *L. monocytogenes*-based ADXS11-001. She received 3 intravenous doses of 1×10^9 CFU ADXS11-001, each followed by oral ampicillin (500 mg) four times daily for 7 days beginning 3 days after administration. The latter two vaccine doses were given 1 and 2 months after a motor vehicle accident that caused multiple fractures requiring internal fixation with an intramedullary rod in the left humerus, a plate with screws in the right humerus, and a lateral ulnar collateral ligament repair with local tissue. The intramedullary rod was removed 10 months later for nonunion. Additional anti-tumor treatment in the 12 months following receipt of ADXS11-001 consisted of monoclonal antibodies directed against the membrane-bound receptor HER3 (ERBB3) and VEGF, external beam radiotherapy, and an experimental PI3K inhibitor.

✉ Douglas A. Drevets
douglas-drevets@ouhsc.edu

1 Section of Infectious Diseases, Department of Internal Medicine, University of Oklahoma Health Sciences Center, 800 Stanton L. Young Blvd, Suite 7300, Oklahoma City, OK 73104, USA

2 Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

3 Advaxis, Inc, Princeton, NJ, USA

4 Medical Services, Department of Veterans Affairs Medical Center, Oklahoma City, OK, USA

5 Present Address: Erie, USA

Thirty-one months after the third and final infusion of ADXS11-001, the patient was given an experimental monoclonal antibody to PD-1. At a scheduled follow-up visit during the first cycle of this new drug, she reported a low-grade fever lasting 1 week as well as confusion. She denied neck pain, neck stiffness, headache, sinus pain, sore throat, nausea, vomiting, rash, motor weakness or gastrointestinal symptoms, and was receiving no immune suppressants. Notably, 10 days prior to this episode she had been hospitalized for pneumonia and received levofloxacin and azithromycin. She was admitted to hospital again at which time her initial vital signs included a temperature of 36.4 °C, blood pressure 95/62 mm Hg, heart rate 135 beats per minute and respiratory rate 14 breaths per minute. Physical examination revealed tachycardia, bilateral wheezing, and a patent colostomy. Her sensorium and neurological examination were normal. Initial laboratory evaluation showed an elevated leukocyte count of 14,620 cells/mm^3 with 91% neutrophils, a hematocrit of 26.5%, and a platelet count of 130,000/mm^3. Serum creatinine, AST, ALT, and total bilirubin were normal. A chest radiograph showed metastatic pulmonary lesions, lymphadenopathy, and post-obstructive atelectasis and/or infection.

Blood cultures from both arms yielded Gram-positive rods within 24 h of collection. The organism was identified as *L. monocytogenes* by the VERIGENE® Gram-Positive Blood Culture Test (Luminex Corporation, Austin, TX, USA) and the API®Coryne (bioMerieux, Marcy-l'Étoile, France) test systems. A peripheral blood culture drawn 4 days later also grew *L. monocytogenes*, whereas a blood culture obtained simultaneously from an infusaport did not. The patient was treated with high-dose ampicillin but declined lumbar puncture, brain imaging and further studies. She died 17 days after admission due to complications of the tumor. An autopsy was not performed.

Dietary history obtained from the patient revealed no obvious food source. No other local *L. monocytogenes* infections were reported to the Oklahoma State Department of Health, nor did the patient have known contact with other vaccine recipients. The isolate was sent to the Centers for Disease Control and Prevention (Atlanta, GA, USA) for further analysis. It was compared with a clinical isolate from another person who was also enrolled in a *Listeria*-based immunotherapy trial and had received the same vaccine at a clinical trial site located outside of Oklahoma. The *L. monocytogenes* clinical isolates were indistinguishable by two-enzyme pulsed-field gel electrophoresis (PFGE) pattern combination and were highly related by whole-genome multilocus sequence typing or wgMLST (3 allele differences) (Jennifer C. Hunter, CDC personal communication). In accord, limited genomic DNA sequencing by the vaccine manufacturer confirmed its identity as ADXS11-001. The clinical isolate to which our patient's strain was compared was obtained within two days of infusion. Thus, reversion of this comparator strain to a more virulent phenotype was considered unlikely. Moreover, both clinical isolates contained an identical, large deletion in the *prfA* gene with no expected function in the truncated gene (Lee S. Katz, CDC personal communication).

Recurrent episodes of naturally occuring *L. monocytogenes* infection are not always caused by the same organism. For example, McLauchlin et al. reported 14 cases of recurrent *L. monocytogenes* infections 4 weeks to > 24 months between episodes [7]. However, analysis of paired bacterial isolates by serovar and phage typing suggested that only 7 of 14 cases were caused by identical organisms. The length of time between episodes of two recurrent cases was only 4 weeks suggesting the initial therapy was inadequate. Inadequate therapy was also the presumed cause of *L. monocytogenes* bacteremia by the same organism 3 weeks after cessation of treatment which consisted of only 3 days of antibiotics [8]. In contrast, infections by the same organisms with longer intervals between episodes, e.g., 9 months to > 2years, suggests wild-type *L. monocytogenes* can persist in vivo then cause recrudescent infection, or possibly remain in the environment in quantities sufficient for initiating a second infection [7, 9]. Notably, such cases have been reported in individuals with underlying conditions known to impair host defenses against *L. monocytogenes*, and / or with infection of prosthetic devices [10].

Pathogenicity of *L. monocytogenes* relies in large part upon an intracellular lifecycle marked by listeriolysin O-mediated lysis of phagosomes, cytoplasmic replication, and F-actin-based motility due to ActA that enables cell-to-cell spread [11]. The ADXS11-001 vector targets HPV-associated tumors by secreting an antigen–adjuvant fusion protein consisting of a truncated fragment of the *L. monocytogenes* protein listeriolysin O fused to HPV16-E7 [4, 5]. This bacterium lacks the *prfA* gene, a positive regulator of multiple *L. monocytogenes* virulence factors including the *hly* and *actA* genes, which encode listeriolysin O and ActA proteins, respectively [4, 5]. As a result, the organism is highly attenuated, with pathogenicity reduced by an estimated 10^4 to 10^5 logs compared with its parent strain [5]. Thus, recurrent bacteremias due to vaccine strains of *L. monocytogenes* are novel because these highly attenuated strains are less likely to survive host defenses in vivo. Recurrent bacteremia with wild-type *L. monocytogenes* was recently reported in 3 of 404 (0.74%) cases of non-maternal invasive listeriois in a large national study [3]. By comparison, as of 22 December 2017, 434 subjects had been enrolled in 11 completed or ongoing clinical studies with ADXS11-001 in HPV + tumors [12]. A total of 1259 doses of ADXS11-001 have been administered, most to women with recurrent cervical cancer. The patient described here is the only reported case of delayed

bacteremia, representing 0.23% of the studied population. In addition to our patient, others have reported *L. monocytogenes* bacteremia 12 days after experimental therapy for pancreatic cancer with a different *L. monocytogenes* vaccine vector, known as CRS-207, based on Δ*actA*/Δ*inlB* mutations [13].

Although the intracellular lifecycle of ADXS11-001 is disrupted, other characteristics are intact. For example, it enters antigen-presenting cells via phagocytosis [4]. In addition, entry into endothelial cells is likely intact as this can be accomplished by Δ*prfA L. monocytogenes* mutants, but without triggering leukocyte adhesion similar to wild-type bacteria [14]. In contrast, biofilm formation, a key factor in the ability of *L. monocytogenes* to persist on prosthetic devices in vivo [15], is positively regulated by *prfA* [16]. We hypothesize that intravenously infused ADXS11-001 infected tissues and / or prosthetic material at recent fracture sites in the patient reported here. Because fracture repair suppresses local immune responses [17], they functioned as protected niches in which bacteria could survive. In addition, the finding that the clinical isolate of ADXS11-001 differed by only 3 SNPs from the reference strain suggests it remained dormant in vivo rather than undergoing continuous replication. The patient's infusaport was present at the time of ADXS11-001 infusion and is another possible source of persistent infection. However blood cultures obtained from it were sterile despite positive cultures from peripheral blood.

It is interesting that vaccine strain *L. monocytogenes* remained quiescent through several cancer treatment regimens until anti-PD1 therapy. Treatment with anti-PD1 enhances cytotoxic T-lymphocyte responses against cells expressing tumor antigens [18]. Thus, cells previously infected with vaccine strain *L. monocytogenes* would express tumor antigens and be targeted for lysis, thereby releasing viable intracellular bacteria in a host already at increased risk for listeriosis. Nevertheless, delayed bacteremia has not been documented in any of 37 subjects treated with another *L. monocytogenes* vector (ADXS-PSA) in combination with pembrolizumab, a PD-1 inhibitor, for metastatic castration-resistant prostate cancer [19]. Mouse studies by Becattini et al. show disruption of gut microbiota by antibiotics can cause rapid expansion of low numbers of *L. monocytogenes* present in the gut, and significantly enhances their ability to invade the host [20]. The patient reported here was treated with levofloxacin and azithromycin 10 days prior to her bacteria. Thus disruption of gut microbiota could have triggered recrudescent bacteremia in this patient should she have been harboring ADXS11-001 in the gut. However, ADXS11-001 is administered intravenous and therefore bypasses the intestinal phase of infection. Moreover this patient received oral amoxicillin, which kills ADXS11-001, after it was infused and the organism was not recovered in stool cultures in phase I studies [5]. Thus, the role of gut infection in persistence or recrudescence of ADXS11-001 in vivo seems less likely than does infection of tissues at or around fracture sites or prosthetic devices.

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Compliance with ethical standards

Conflict of interest This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. EF, CM, LS, and DD are not affiliated with, nor received any funding from Advaxis, Inc. AG and MP are current employees of Advaxis, Inc.

Ethics statement The clinical trial under which the patient received experimental immunotherapy and subsequent review of her records were done with the approval of the Institutional Review Board at OUHSC.

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