Staphylococcus aureus Panton-Valentine Leukocidin Targets Muscle Tissues in a Child with Myositis and Necrotizing Fasciitis

Deborah Lehman,1,4* Ching Wen Tseng,2,3,4* Samantha Eells,1,7 Loren G. Miller,7 Xuemo Fan,1 David O. Beenhouwer,5,6 and George Y. Liu1,2,4

1Division of Pediatric Infectious Diseases, 2Immunobiology Research Institute, and 3Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Departments of 4Pediatrics and 5Medicine, David Geffen School of Medicine, University of California, Los Angeles, and 6Division of Infectious Diseases, Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, and 7Division of Infectious Diseases and 8Los Angeles BioMedical Research Institute at Harbor–University of California, Los Angeles, Torrance, California

The incidence of myositis has been increasing since the advent of the epidemic of community-associated methicillin-resistant Staphylococcus aureus infection, and Panton-Valentine leukocidin has been implicated as a factor contributing to more-severe muscle injury. We report a case of severe myositis accompanying septic osteomyelitis and necrotizing fasciitis caused by a Panton-Valentine leukocidin–positive S. aureus strain. Immunostaining showed strong binding of the Panton-Valentine leukocidin toxin to necrotic muscle tissues.

Staphylococcus aureus myositis, pyomyositis, and necrotizing fasciitis are severe but uncommon infections in the United States [1]. Epidemiologic studies suggest that the incidence of these infections is increasing [2, 3]. It has been proposed that Panton-Valentine leukocidin (PVL), a toxin that induces cytolysis of human phagocytes, could play a major role in pathogenesis [3]. PVL has been reported to be associated with more-severe cases of myositis [3], and recently, PVL has been shown to contribute to more-severe muscle injury in mice [4, 5].

We describe a case of osteomyelitis, septic arthritis, myositis, and necrotizing fasciitis caused by a PVL-positive methicillin-susceptible S. aureus (MSSA) strain. PVL immunostain of the patient’s biopsy specimen unexpectedly revealed strong binding of the PVL toxin to necrotic and degenerative skeletal muscle tissues.

Case report. A previously healthy 6-year-old boy presented to his pediatrician with 2 days of fever (maximum temperature, 38.9°C). The week before presentation, the patient had noted a pustule over his right olecranon, which grew to ~3 cm in diameter over 4 days. A small amount of serosanguinous fluid was manually expressed from the pustule, and the patient initiated oral amoxicillin-clavulanate therapy. Two days after the initiation of antibiotic therapy, he developed left upper extremity pain and spiking fevers. He had no trauma to the area and no respiratory or gastrointestinal symptoms.

The patient’s history was unremarkable for any infections or hospitalizations, and his immunizations were up to date for his age. His family history was significant; his father had recurrent skin pustules of unknown etiology but no other infections or autoimmune conditions.

The patient’s physical examination was significant for a temperature of 39.3°C, pulse rate of 148 beats per min, respiratory rate of 24 breaths per min, and blood pressure of 122/78 mm Hg. His oxygen saturation was 100% while breathing room air. An examination of the patient’s extremity showed warmth and swelling around the left shoulder. He had significant discomfort with any motion of the shoulder but had full range of motion of his elbow and wrist, without tenderness. His motor and sensory examination was intact in radial, median, and ulnar nerve distributions. The patient had a healing lesion over his contralateral olecranon, without erythema or drainage.

Laboratory findings at hospital admission included an elevated white blood cell count (23,700 cells/mm³), with 72% neutrophils, 14% band forms, and 3% mononuclear cells. The patient’s hemoglobin level was 10 g/dL, and his platelet count was 294,000 platelets/mm³. A small amount of serosanguinous fluid was manually expressed from the pustule, and the patient initiated empirical intravenous vancomycin and cefotaxime. Two days after the initiation of antibiotic therapy, he developed left upper extremity pain and spiking fevers. He had no trauma to the area and no respiratory or gastrointestinal symptoms.

Cultures of both blood and joint fluid specimens grew S. aureus susceptible to oxacillin, clindamycin, trimethoprim-sulfamethoxazole, gentamicin, and vancomycin, with resistance to penicillin.

On the first day after surgery, the patient had an acute respiratory decompensation and required endotracheal intubation. A chest radiograph revealed bilateral nodular densities...
and areas of wedge-shaped pulmonary consolidation consistent with pulmonary emboli. The result of a follow-up blood culture on hospital day 3 was negative. Because of persistent fevers and left arm pain with swelling, the patient required additional upper extremity debridement procedures on the second and fifth hospital days. *S. aureus* was recovered from joint fluid, muscle, and periosteum specimens obtained during both surgical interventions. Gram-positive cocci were noted during histological examination of the deltoid muscle. Anatomic pathology specimens confirmed marked myositis with severe myofiber degenerative changes, necrosis, myophagocytosis, and acute fasciitis. Antibiotics were changed to oxacillin and gentamicin, and a follow-up chest radiograph on the fourth hospital day showed areas of decreased attenuation at the lung bases, radiologically consistent with small abscesses. Transthoracic echocardiogram findings were negative for vegetations, and doppler examinations were negative for deep venous thromboses in both the patient’s upper and lower extremities.

The patient completed 6 weeks of intravenous therapy with oxacillin, and follow-up imaging showed a pathologic fracture of the humerus with callus formation. He recovered excellent function of his left upper extremity, without functional deficit, and full range of motion.

**Methods.** Genotyping of *S. aureus* strains was performed by pulsed-field gel electrophoresis with *SmaI* digestion, according to a published protocol [6]. Toxin genes encoded by the *S. aureus* strain were identified by polymerase chain reaction (PCR) [7, 8]. Genomic DNA isolated from well-characterized *S. aureus* strains from the National Institutes of Health Network for Antibiotic Resistance in *Staphylococcus aureus* ([http://www.narsa.org](http://www.narsa.org)) were used as controls for PCR. Rabbit antisera against PVL toxin were generated using a protocol described elsewhere [5]. The serum samples were tested against purified α-toxin, γ hemolysin, and overnight culture supernatant from *S. aureus* strain Newman (ST8 clonal lineage) on a Western blot and did not show cross-reactivity.

Hematoxylin and eosin stains were performed on formalin-fixed paraffin embedded tissues. The tissue slides were stained with rabbit polyclonal anti–PVL-F antibody and rabbit polyclonal anti–α-toxin antibody (Sigma). After overnight incubation at room temperature, the slides were washed and incubated in the presence of diluted secondary antibody conjugated to horseradish peroxidase (Cell Signaling). After overnight incubation, the slides were washed with phosphate-buffered saline and mounted with mounting media (Invitrogen). An Institutional Review Board exemption was obtained for performance of the immunostains.

**Results.** Genotyping identified the patient’s MSSA iso-
late as belonging to the clonal lineage ST8, pulsed-field type USA300. Consistent with a previously published toxin profile for USA300 [2], the genome of the MSSA isolate did not harbor genes for enterotoxins A–J (sea, seb, sed, see, seg, sej, sei, ser, and sej), or tst (the toxic shock syndrome toxin gene) but encoded and expressed both LukS-PV and LukF-PV, as determined by PCR and Western blot analyses (data not shown).

Hematoxylin and eosin stain of biopsy tissue samples from the upper extremity debridement showed presence of gram-positive cocci and marked acute inflammation involving both fascia and muscle tissues, causing significant necrosis (Figure 1A). Because of the reported association of PVL with severe myositis [3], we evaluated PVL expression in biopsy tissue specimens with use of specific antiserum against LukF-PV. Skeletal muscle fibers displayed degenerative change, myophagocytosis, and myofiber necrosis, frequently seen in a segmental pattern (Figure 1B and 1C). However, no apparent regenerating fibers or interstitial fibrosis were noted.

Immunoperoxidase stains for PVL showed focal positivity in segments of skeletal muscle fibers corresponding to segments with degenerative changes and/or myofiber necrosis. In addition, there appeared to be some immunostains in edematous interstitial tissue, although no immunostains were noted in vascular endothelial cells, smooth muscle cells, adipocytes, erythrocytes, or leukocytes.

To ensure specificity of the rabbit antiserum used to identify the presence of PVL, we examined Streptococcus pyogenes–infected tissue and an age-matched noninfected muscle biopsy specimen. No immunoreactivity for PVL was identified in skeletal muscle fibers, including myofibers with segmental degeneration and/or myofiber necrosis, from a patient with S. pyogenes infection or in a noninfected muscle tissue specimens from an 8-year-old girl. Immunostaining of the patient’s tissue with pre-immune serum (data not shown) or antibody to S. aureus α-toxin also showed no reactivity (Figure 1B).

Discussion. In the decade since the beginning of the epidemic of community-associated methicillin-resistant S. aureus (MRSA) infection, much attention has been focused on which factors could contribute to more-severe S. aureus infection. Many believe that community-associated MRSA isolates can cause more-severe infection, because they have acquired novel virulence genes, particularly PVL [9, 10], which in clinicopepidemiologic studies has been linked to more-severe S. aureus infection of the lung, bone, muscle, and fascial tissues [2, 3, 11–14]. PVL is thought to contribute to tissue injury by inducing apoptosis and cytolysis of granulocytes [15, 16]. In mice and rabbits, injection of purified PVL toxin leads to induction of inflammation and necrosis [17, 18].

The role of PVL in S. aureus pathogenesis is controversial; some animal investigations that used PVL knockout MRSA strains showed no effect of PVL on infection severity [19–22], and other studies suggest that PVL is a major virulence determinant [4, 18]. Further fueling the debates is the finding that PVL is a relatively weak cytolytic toxin, compared with other secreted S. aureus toxins, such as α-toxin, which causes significant tissue damage during infection [19], and it is difficult to argue how such a weak toxin could have a major role in the epidemic when other S. aureus toxins (eg, α-toxin) have much stronger cytolytic activity [19].

Our finding that PVL binds to necrotic or degenerating muscle tissue offers new insight for pathogenesis of S. aureus deep-tissue infections. PVL-positive strains have been associated with more-severe cases of human myositis [3], and PVL-positive S. aureus strains preferentially cause myositis in individuals with osteomyelitis [11]. Furthermore, we and others have independently reported, using different mouse models with wild-type and PVL knockout community-associated MRSA clones, that PVL contributes to more-severe muscle pathology [4, 5]. These findings taken together suggest that PVL plays an active role in human myositis.

From a mechanistic perspective, we found that PVL did not stain neutrophils or leukocytes above background levels, but there was marked staining of select muscle bundles and interstitial fluid. Because of technical limitations, staining of other host cells could not be ruled out. Most published reports have credited cytolysis or apoptosis of phagocytes to be the primary mechanism of PVL-mediated tissue injury. However, cytolysis of phagocytic cells has only been shown with a high concentration of purified PVL toxins [15], and the use of live isogenic strains of PVL-positive and PVL-negative S. aureus failed to demonstrate that PVL mediates neutrophil lysis at physiologic concentrations [21].

In light of this new data, the current model by which PVL induces injury by killing of phagocytic cells may require careful re-evaluation. Our results suggest a possible direct effect of PVL on human muscle tissue. Although our investigation is limited by sample size, our findings prompt the questions of which other cells interact with PVL and whether these interactions contribute significantly to pathogenesis of severe S. aureus infections.

Acknowledgments
We thank Dr. Moshe Arditi for peer review of the manuscript.

Potential conflicts of interest. All authors: no conflicts.

Financial support. Burroughs-Wellcome Career Award and National Institutes of Health (AI074832 to G.Y.L.).

References
3. Panzaraj PS, Hulten KG, Gonzalez BE, Mason EO Jr, Kaplan SL. Infective pyomyositis and myositis in children in the era of community-


