Panton–Valentine leukocidin (PVL) Role in the Induction, Maintenance and Local Extension of Community-Associated Methicillin-Resistant Staphylococcus aureus (CA-MRSA) Rabbit Osteomyelitis

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The role of PVL in CA-MRSA pathogenicity remains controversial and might depend on the infection site and/or experimental model.

Osteomyelitis has long been recognized as a major clinical syndrome of invasive *S. aureus* disease.

The impact of PVL on the course of acute osteomyelitis in children and young adults was already suspected during the pre-antibiotic era (Specific immunity in acute staphylococcal osteomyelitis. Valentine and Butler Lancet 1939;1: 973-8)

was recently readdressed in the era of CA-MRSA (CE Bocchini et al. Pediatrics 2006; 117:433-40
Study Objective

◆ Assess the specific role of PVL in a CA-MRSA rabbit osteomyelitis model

◆ By comparing the outcomes of infections caused by
  ✤ PVL-positive MRSA USA 300: LAC
  ✤ PVL-negative isogenic strain: LACΔ pvl
    (kindly provided by Frank R. DeLeo)
Rabbit osteomyelitis model
(Norden’s model J Infect Dis 1970)

- New Zealand white rabbits
- Intramedullary injection of a sclerosing agent (0.1 mL of 3% sodium tetradecyl sulfate) into the tibia, followed by 0.2 mL of inoculum
- Inocula: 8×10⁵ (low) or 4×10⁸ (high) CA-MRSA CFU (cultured in CCY and diluted in PBS)
- On D7 and D28, rabbits were sacrificed
  - macroscopic findings: noted and photographed,
  - infected tibias were removed and bacteria in crushed bones were counted.
  - Serum samples: Anti PVL antibody and CRP titers
Imaging and histopathological examinations

- **Low inoculum**: Serial MRI (Philips INTERA 1.5T) performed on 6 rabbits (D7, D14, D21)

- **High inoculum**: Plain film +MRI + histopathological examination of 6 rabbits at the time of sacrifice (D7 or D28)
![Bar chart showing the percentage of infected animals with and without pvl at different time points.](image)

Results
Low Inoculum (8 x 10^5 CA-MRSA CFU)

- **D7 + D28**
  - % infected animals
  - n = 19

- **p = 0.04 vs LAC D pvl**

*Fisher’s Exact test*
Results
Low Inoculum (\(8 \times 10^5\) CA-MRSA CFU)

![Graph showing Log \(_{10}\) CFU/g of bone with error bars and legend indicating statistical significance.]

- **D7**:
  - LAC: n = 10, p = 0.03 vs. D28*
  - LAC D pvl: n = 10

- **D28**: (Legend information for comparison)

* Mann-Whitney non-parametric U-test
MRI  PVL+

D7  D14  D21  D28

4.6 log_{10} CFU/g
MRI PVL

D7
D14
D21

Control

6.0 log_{10} CFU/g

D28
Results
High Inoculum (4x10^8 CA-MRSA CFU)

<table>
<thead>
<tr>
<th></th>
<th>D7</th>
<th>D28</th>
</tr>
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<tbody>
<tr>
<td>LAC</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>LAC D pvl</td>
<td>100</td>
<td>86</td>
</tr>
</tbody>
</table>

% infected animals

- n = 12
- n = 11
- n = 7
Results
High Inoculum (4 x 10^8 CA-MRSA CFU)

![Graph showing Log 10 CFU/g of bone over time.]

- **D7 vs D28**
  - LAC: n=12, P = 0.004 vs D28*
  - LAC D pvl: n=10

- **D28**
  - LAC: n=7

* Mann-Whitney non-parametric U-test
Results

High Inoculum (4 x10^8 CA-MRSA CFU)

Macroscopic findings

- % infected animals:
  - 100%
  - 67%
  - 0%
  - 60%
  - 57%
  - 7%
  - 57%
  - 9%

- LAC Δ pvl

- Day 7:
  - LAC: 100%
  - LACΔpvl: 60%

- Day 28:
  - LAC: 60%
  - LACΔpvl: 57%

- P values:
  - P=0.001 vs LAC
  - P=0.03 vs LAC

- Bone marrow involvement
- Abnormal bone architecture
- Muscle or joint involvement

Images showing bone marrow and abnormal bone architecture involvement.
High Inoculum \((4 \times 10^8 \text{ CA-MRSA})\)
Radiographic and histological findings in a PVL\(^+\)-infected rabbit on D28

- Deformation and widening of the entire diaphysis
- Bone Abscess
- Sequestrum
High Inoculum

MRI and histological findings in a PVL⁺-infected rabbit on D28

Soft tissue Abscess 3x2x1(cm)
High Inoculum
MRI and histological findings in a PVL$^+$ - infected rabbit that died on D8

Abscess in the joint cavity

Pyomyositis
Distribution of C-reactive protein (High inoculum)
Distribution of anti-PVL antibody levels in sera

![Graph showing distribution of anti-PVL antibody levels](image)
Conclusion

- Our results showed that PVL could contribute to the pathogenesis of early and late phase of CA-MRSA rabbit osteomyelitis by enabling:
  - Better bacterial persistence (low inoculum)
  - Local extension during the infection’s early phase (high inoculum)
- Concordant with clinical studies

- Differences with previous experimental explorations of the role of PVL could be explained by
  - Location of infection
  - The experimental model used: similar to the human situation; allows evaluation at different times post-infection
  - Selection of clinically relevant experimental readouts

- This model may be suitable to select optimal therapies for PVL+CA-MRSA osteomyelitis