Video Article

Subcutaneous Infection of Methicillin Resistant Staphylococcus Aureus (MRSA)

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Abstract

MRSA is a worldwide threat to public health, and MRSA skin and soft-tissue infections now account for more than half of all soft-tissue infections in the United States. Among soft-tissue infections, myositis, pyomyositis, and necrotizing fasciitis have been increasingly reported in association with MRSA arising from the community. To understand the interplay between MRSA and host immunity leading to more severe infection, the availability of animal models is critical, permitting the study of host and bacterial factors. Several infection models have been introduced to assess the pathogenesis of *S. aureus* during superficial skin infection. Here, we describe a subcutaneous infection model that examines the skin, subcutaneous, and muscle pathologies.

Video Link

The video component of this article can be found at https://www.jove.com/video/2528/

Protocol

1. Preparing the MRSA for Infection (two Days Prior to Infection)

1. Inoculate a loopful of MRSA from a stock culture to a blood agar (Trypticase Soy Agar (TSA)) plate.
2. Check the hemolysis phenotype (a clear zone around each colony) on the blood agar plate.
3. Pick a colony that has a hemolytic phenotype that is consistent with other experiments.
4. Inoculate the colony into 3 mL Todd Hewitt Broth (THB),with the appropriated antibiotic when necessary, in a 15 mL snap-capped tube.
5. Incubate at 37°C overnight with shaking at 220 rpm.

2. Preparing the Mice for Infection (one Day Prior to Infection)

1. Depending on the size of the mice, shave the fur off a 3 x 4 cm area on the back of mice.
2. Apply ~5 mm^3 hair remover cream (purchased from a local drug store) to the shaved area.
3. Allow the hair remover cream to incubate on the skin surface for approximately 1 min.
4. Wet paper towels with ddH_2O.
5. Wipe off the hair remover cream with the wet paper towel.

3. Preparing the MRSA for Infection (on the Day of Infection)

1. Dilute the overnight bacterial culture at 1:500 to 1:1000 with 10 mL of pre-warmed THB, in a 50 mL screw-capped tube.
2. Incubate at 37°C for approximately 2.5 hours with shaking at 220 rpm, until A_{540} reaches 2.5.
3. Collect MRSA by centrifugation at 3,225 x g for 10 min at 4°C.
4. Discard the supernatant.
5. Resuspend the bacterial pellet in 10 mL Dulbecco's phosphate buffered saline (DPBS).
6. Repeat steps 3 and 4.
7. Resuspend the bacterial pellet in DPBS at a desired concentration.
8. Serially dilute the bacterial suspension from 10^1 to 10^8.
9. Plate the diluted bacterial suspension onto blood agar plates.
10. Incubate the plates at 37°C overnight.
11. Observe and record the hemolytic phenotype of the inocula.
12. Count the Colony Forming Units (CFU) number on the plate.

13. Calculate the inocula based on the CFU number on the plate.

4. Subcutaneous Infection of MRSA (on the Day of Infection and 3 Days Post-infection)

1. Inject subcutaneously 100 µL bacterial suspension in the shaved area.
2. Observe the animals for 3 to 5 hours after injection to make sure the mice are alive.
3. Observe the lesion on the back of the animals daily and record the lesion area daily when it is needed. Lesion observation should include recording of lesion size and morphology. Both skin and muscle lesions are quantitated by multiplying the length and width of the lesion. Irregularly-shaped lesions need to be broken down into smaller symmetrical pieces, and each piece measured by the same method. Lesion measurements may also use a computer-assisted histomorphometric assessment program (ImageJ; open-source available from the NIH at http://rsb.info.nih.gov/ij/) [2].
4. Sacrifice the animals on day 3 post-infection by inhalation of isoflurane followed by cervical dislocation.
5. Observe and record the lesion on the skin.
6. Nick the skin with a pair of sterile scissors.
7. Peel the skin off carefully and observe and record the lesion on the muscle.
8. Excise the lesion and approximately 2 - 5 mm of the surrounding area.
9. Collect spleen and kidneys.
10. Homogenize the tissues using a tissue homogenizer or by repeated application of a plunger from a 1 mL syringe to a microfuge tube containing the tissue and 100 µL of DPBS.
11. Add 900 µL of DPBS to each tube.
12. Vortex the samples for 5 min.
13. Serially dilute the suspension with DPBS 10^1 to 10^6.
14. Plate the diluted suspensions on THA plates.

5. Representative Results:

1. Immediately after each subcutaneous injection, a bleb will be observed on the skin surface when infection is done correctly (Figure 1A). If no bleb is observed on the skin surface, the injection may be too deep, and this may affect the outcome of the infection (lesion size, CFU).
2. The bacterial suspension needs to be serially diluted and the CFU need to be examined on blood agar plates for each experiment. This step will yield important information: 1) whether the inocula have homogeneous phenotype; 2) the exact viable bacterial count for the infection.
3. The lesions and bacterial survival may be assessed at various time points post-infection. Here, we examined the infection on day 3 post-infection. The lesion sizes are analyzed on the skin surface (Figure 1B: 1x 10^9; Figure 1D: 1 x 10^7 CFU) and on the muscle surface (Figure 1C: 1x 10^9; Figure 1E: 1 x 10^7 CFU). The lesion area equals length (mm) x width (mm) may be measured for assessing the tissue damage (Figs. 2 A and B). This result may help determine the extent of tissue damage caused by a given virulence factor. Additionally, the lesions are excised and homogenized in DPBS, and plated to quantitate the CFU number (Figure 2C), which indicates the viability of the bacteria at the infection site.

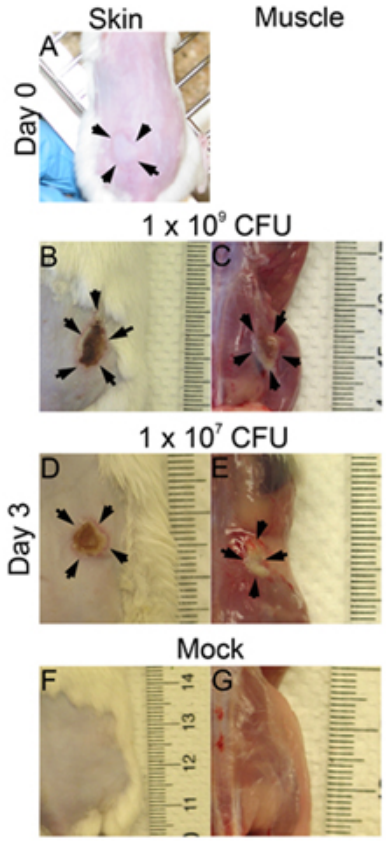

Figure 1. The skin and muscle lesions. CD1 mice were inoculated subcutaneous on one flank with MRSA (LAC). Pictures were taken at time 0 and day 3 post-infection. (A) Time 0 post-infection (p.i.), (B)-(G) Day 3 p.i.; B, D. and F: skin lesions; C, E, and G: muscle lesions; B and C: 1x 10^9 CFU infected; D and E: 1x 10^7 CFU infected; F and G: mock.

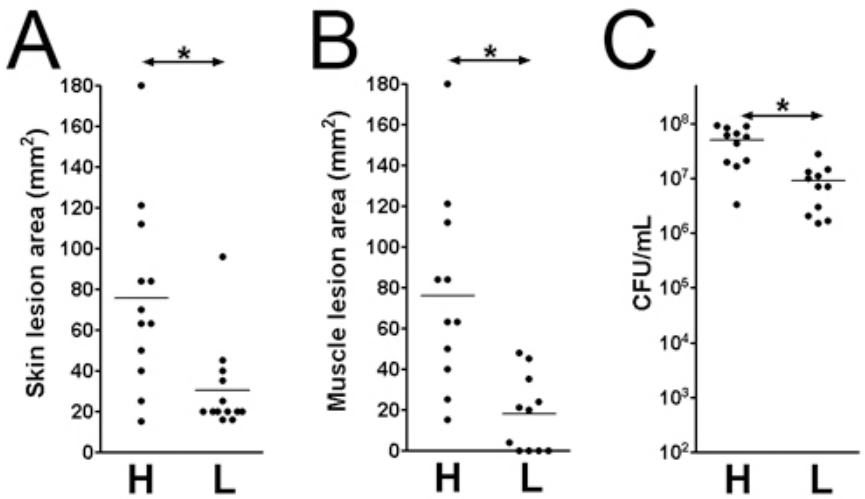

Figure 2. Skin lesion size and viable bacteria count at the infection site on day 3 post-infection. Skin and muscle lesions on MRSA (LAC) infected CD1 mice. (A) Skin lesion size. (B) Muscle lesion size. (C) Total tissue CFU. H: 1 x 10^9 CFU inocula; L: 1 x 10^7 CFU inocula. *: $p < 0.05$, Mann-Whitney test.

Discussion

1. The murine skin and soft tissue infection model is a powerful tool for *in vivo* virulence assessment of a pathogen. The pathogenicity of *S. aureus* in skin and soft tissue infection may vary depending on a number of parameters. These include inoculum size, bacterial growth phase, depth of inoculation, age of mice, and the mouse genetic background [7, 8]. When examining the virulence function using this model, it is critical to control for these parameters to ensure that results will be consistent. We have studied MRSA infection of the skin in several strains of mice, including SKH1 (hairless mice), C57Bl/6, BALB/c, and CD1. Infection outcomes vary in the different strains [7], consistent with other reports that the genetic makeup of the mouse is an important determinant of the severity of MRSA infection [1]. In this experiment, we utilize

CD1 mice because this mouse strain has been shown to be well-suited for studies of *S. aureus* virulence factors [5] and is less expensive than other strains.

2. **To ensure that the results of experiments are consistent, the colony picked for infection must be checked for its hemolysis phenotype prior to each experiment.** The β-hemolytic phenotype on sheep blood agar (a clear zone around the colony) reflects expression of α-toxin by the *S. aureus* isolate. This step is essential because spontaneous mutation of a *S. aureus* global regulator has been reported to occur, resulting in depressed α-toxin expression [6]. *S. aureus* lacking α-toxin expression exhibits a less virulent phenotype compared to isogenic strains that express α-toxin [4].
3. After shaving the fur off the back of mice, apply approximately 5 mm^3 sized hair remover cream (purchase from local drug store) to the shaved area. This step will keep the back of mice free from hair for 5 to 7 days. Several types of hair remover cream will work; however, it is important to choose one that induces the least amount of skin irritation. We recommend hair remover creams with baby oil.
4. When injecting the bacterial suspension, a bleb will be observed on the skin surface (Figure 2A). When an injection is too deep (no bleb observed), the suspension may be inoculated into the muscle, which will affect the infection outcome because of the wrong infection site, and lesion and CFU recovery from the subcutaneous tissue for that mouse will be different.
5. In addition to lesion size and CFU count, dependent variables may also be examined to assess virulence. These include bacteria dissemination, serum cytokine levels, infection site cytokine and chemokine levels, PMN recruitment, and histology [3, 6].
6. For histology and immunochemistry analysis, infected tissue may be excised and fixed in 10% formalin overnight. When excising the infected tissues, 2 - 5 mm healthy tissue sounding the lesion needs to be cut with the lesion. This will assure that no deep pathologic tissues are omitted.

Disclosures

No conflicts of interest declared.

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