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In vivo Analysis of Neutrophil Infiltration during LPS-induced Peritonitis

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[Abstract] Bacterial lipopolysaccharide (LPS) is present in the outer membrane of Gram-negative bacteria and functions as pathogen-associated molecular pattern (PAMP) (Whitfield and Trent, 2014). LPS therefore is a potent activator of inflammatory responses leading to cytokine release and neutrophils recruitment. The lipid A moiety of LPS activates the complex consisting of the LPS binding protein (LBP), CD14, MD-2 and Toll-like receptor 4 (TLR4) and the non-canonical inflammasome-linked caspases-4, 5 and 11, which in turn activate the canonical NLRP3 inflammasome (Shi *et al*., 2014; Hagar *et al*., 2013; Kayagaki *et al*., 2013; Hoshino *et al*., 1999; Poltorak, 1998; Nagai *et al*., 2002; Park *et al.*, 2009; Ratsimandresey *et al*., 2013). In particular, the cytokine interleukin (IL)-1β produced in response to inflammasome activation has a crucial role in neutrophil recruitment through promoting neutrophil adhesion and migration (McDonald *et al*., 2010).This protocol allows studying of inflammatory response induced by LPS that affect neutrophil infiltration by tracking myeloperoxidase (MPO) activity *in vivo* (de Almeida *et al*., 2015).

Materials and Reagents

1. Insulin syringes (Thermo Fisher Scientific, catalog number: 14-841-31)
2. 0.22 µm filters
3. C57BL/6 mice, typically of 8-12 weeks old mice (male or female)
4. LPS *E.coli* 0111:B4 (Sigma-Aldrich, catalog number: L2630-100MG)
5. Dulbecco's phosphate-buffered saline (DPBS) (Corning, catalog number: 21-030-CV)
6. XenoLight RediJect inflammation probe (PerkinElmer, catalog number: 760535)
7. Luminol sodium salt (Sigma-Aldrich, catalog number: A4685)
8. Isofluorane (Henry Schein, Isothesia™, catalog number: 10014450)
9. 5 mg/ml LPS (see Recipes)
10. 20 mg/ml luminol sodium salt stock solution (see Recipes)

Equipment

1. Anesthesia machine (VetEquip, model: 901808) or similar anesthesia equipment
2. Rechargeable trimmer (Braintree Scientific, catalog number: VLP-323 75)
3. Scale (Kent Scientific, catalog number: SCL66110)

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4. Biosafety cabinet
5. IVIS spectrum (PerkinElmer, model: 124262) or a comparable luminescence imaging equipment

Software

1. Living Image software (PerkinElmer)

Procedure

1. Two days before the LPS intraperitoneal injection, place mice in the anesthesia machine and once the mice are anesthetized shave abdominal area with a trimmer, as fur quenches the luminescence signal (Figure 1).

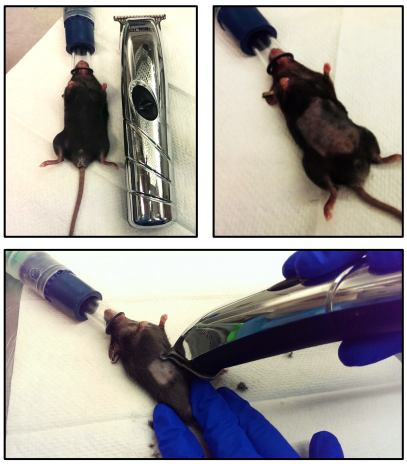

Figure 1. A representative picture of mice under anesthesia getting their abdominal area shaved with a trimmer

2. Weigh mice.
3. In the day of the experiment dilute LPS in DPBS and prepare syringes for injection.
4. Intraperitoneally inject mice with 2.5 mg/kg of LPS or the same volume DPBS for the control group (injection volume approximately 200 µl).
5. 3 h later intraperitoneally inject mice with 200 mg/kg of XenoLight Rediject inflammation probe or 200 mg/kg luminol sodium salt (injection volume approximately 200 µl).
6. Place mice in the IMPAC6 anesthesia chamber attached to the IVIS spectrum.
7. Transfer mice to the IVIS spectrum and place mice abdomen facing up into the chamber and position each nose inside the cone that delivers the isofluorane (Figure 2).
8. Start imaging anesthetized mice 10 min post XenoLight Rediject inflammation probe injection with a 5 min exposure capturing *in vivo* bioluminescence generated by the activity of MPO as a

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marker for infiltration of neutrophils. In order to imagine 5 mice select field of view D and select 1.5 cm subject height (Figures 2 and 3) (Gross *et al*., 2009; Tseng and Kung, 2012).

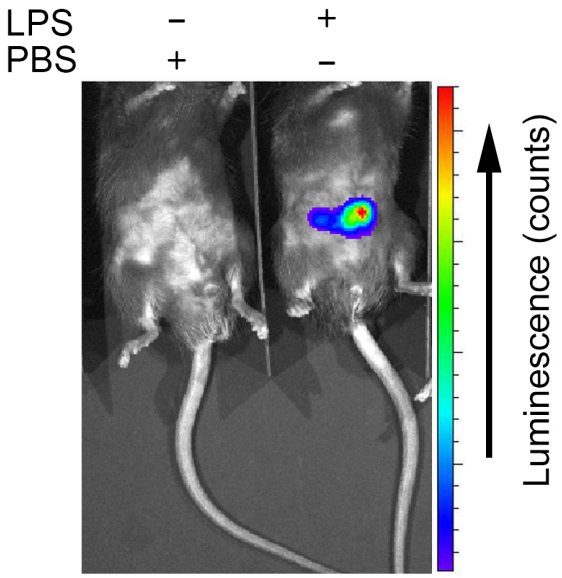

Figure 2. A representative example of *in vivo* imaging in 8 wk of age male C57BL/6 mice after i.p. injection of PBS (left) or LPS (2.5 mg/kg body weight) (right)

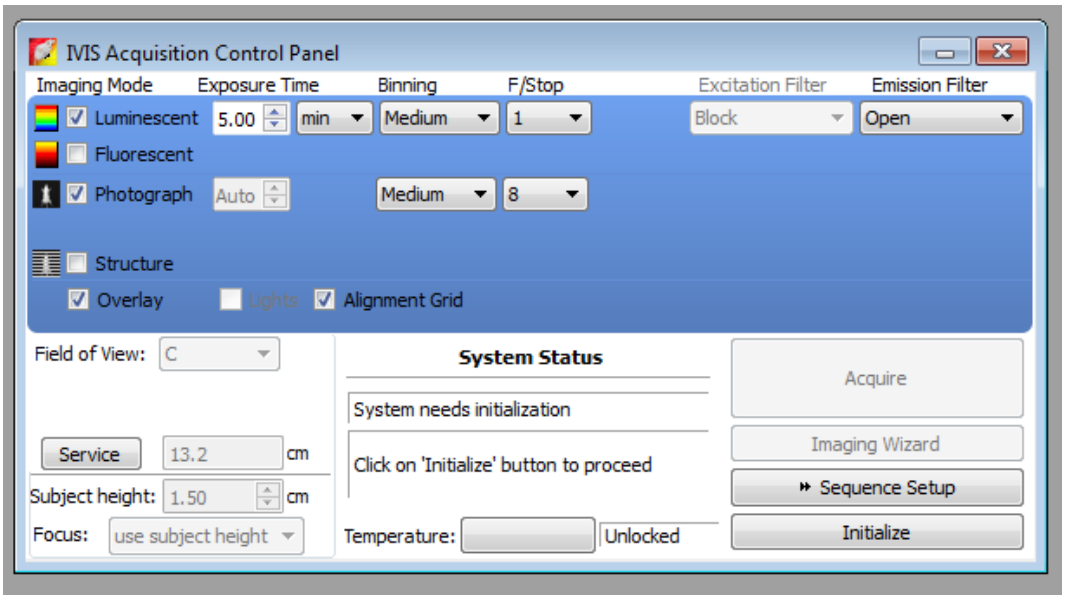

Figure 3. Screen capture image of the IVIS acquisition control panel

9. Quantify the MPO signal using the Living Image software. First select region of interest (ROI) using ROI tools and choose to automatically draw measurement ROIs and perform ROI analyses to measure photon radiance. Also measure background ROI and subtract from your ROI measurement. Use average radiance to plot your graph.

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Recipes

1. 5 mg/ml LPS
   Dilute LPS in DPBS.
   Filter-sterilize with a 0.22 µm filter and aliquot stock solution at -80 °C.
2. 20 mg/ml luminol sodium salt stock solution
   Prepare 20 mg/ml luminol sodium salt stock solution in DPBS.
   Note: Luminol sodium salt for injection has to be prepared fresh each time in DPBS (20 mg/ml), filter-sterilize with a 0.22 µm filter and protected from light until use.

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