Animal Use Protocol (AUP) Amendment Application

Use this form to request an amendment for your current Institutional Animal Care and Use Committee (IACUC) approved AUP. Complete items #1-3. Submit the AUP Amendment Application electronically to the Compliance Office (iacuc@ucr.edu), 207 UOB.

1. General Information:

AUP #:	20150011BE
AUP Title:	Mouse mucosal immunity
Principal Investigator:	David Lo
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Submission Date:	03/23/2016

2. Type of amendment request (check all that apply):

mouse	Animal species/strain
150	Animal number
no	Additional vivarium space (contact the Campus Veterinarian, 2-5845, if additional space will be needed)
colony	Animal source
x	Animal use procedure
	Animal care procedure
Infectious bacteria (*S. aureus*, UAMS-1)	Use of potentially hazardous substances ** (see below)
Pain and Distress Category - 2	Pain and Distriess (please specify in writing):

** Changes in the use of potentially hazardous substances must include an updated *Hazardous Materials Information* form (http://www.ora.ucr.edu/vet/Forms/AUP.doc section 8.0).

3. In narrative form briefly describe and justify the amendment request.

Be sure to include any related changes in:
- animal husbandry or housing (including environmental enrichment),
- anesthesia monitoring
- post-surgical monitoring

For changes in procedures, always address whether or not the consideration of adverse effects in the original protocol (section 5.0) thoroughly covers changes described in the amendment.

This is a new procedure for intravital imaging of a catheter infection model revised in this version to use a non-MRSA strain, using the multiphoton microscope in V3 (revised 04/04/16, and an SOP for BSL-2 procedures is also attached).

14. Induction of biofilm formation and intravital imaging of infiltrates in ear pinnae

To study the infectious complications of surgical device implantation and infection by biofilm-forming microbes, we will implant a sterile catheter material and infect with Staphylococcus aureus. At various times after implant and infection from days 1 through 8, mice will be used for intravital imaging under anesthesia with the Nikon A1R multiphoton microscope. Our protocol is based largely on published studies by Kielian et al. (e.g., Thurlow et al., J Immunol 2011; 186:6585-6596) but adapted to use on an ear implant model instead of a flank implant to enable intravital imaging through the ear pinnae, and will be conducted in collaboration with the Kielian lab.

Methods:

The *Staphylococcus aureus* strain UAMS-1 is a non-MRSA clinical isolate. We will in some cases transform it to harbor a plasmid (pCM11) expressing the fluorescent protein EGFP. While this bacteria is a clinical isolate and can infect humans, it can be handled under conventional BSL2 protocols, and the microscope objective and other equipment will be disinfected using 70% ethanol. **For reference, it should be noted that Staphylococcus is a common commensal microbe, asymptomatically colonizing the nose, respiratory tract, and skin of humans, and is not generally pathogenic unless they produce toxins that promote infection. Our studies are aimed at studying the impact of bacterial biofilm formation on implanted medical devices.**

Implantation of the catheter will be done under ketamine/xylazine anesthesia (80mg/ml ketamine, 12 mg/ml xylazine; 1ml/kg body weight IP). **Depth of anesthesia will be frequently monitored by signs of spontaneous movement, and absence of response to foot pinch, at which point surgery can begin.** The ear will first be prepared by using Nair to remove skin hair **followed by three alternating wipes of betadine and alcohol**. Then a scalpel will be used to cut into the proximal portion of the ear pinna (a line parallel to the head), and using forceps, a small cavity will be made by separating the ear skin from underlying connective tissue. A 3-5mm length of catheter material will be inserted into the cavity and the implant will be gently pressed in place and the wound closed, and sealed in place with VetBond tissue adhesive. Infection will be accomplished either by placing 10^3 CFU *S. aureus* in agar in the lumen of the catheter prior to implantation, or by transcutaneous injection of 10^3 CFU *S. aureus* into the lumen of the implanted catheter. Apart from post-surgical wound pain, the animals should not suffer from pain from the implant. **Recovery from surgery will be monitored continuously for the next 30-60 minutes until the mouse is spontaneously moving around the cage. Post surgery animals will be housed in the BSL-2 room.** Post-operative analgesia will be provided by Carprofen (5/mg/kg SQ administered every 12-24 hours), Meloxicam (5mg/kg SQ, administered every 12-24 hours) or Flunixin (2-2.5 mg/kg SQ administered every 12-24 hours)

Animal welfare will be monitored daily for weight loss, lethargy, signs of external wound infection or irritation. Mice will be euthanized if they show signs of stress such as loss of >15% body weight, or if the implant site shows signs of re-opening, or open wound infection (since this is specifically an implant infection model, not a cutaneous infection).

At various times after implantation, animals will be anesthetized using ketamine/xylazine anesthesia (80mg/ml ketamine, 12 mg/ml xylazine; 1ml/kg body weight IP), and the ear will be placed on a plastic platform held in place by tape sticky side up to hold on to the ear from below. This will hold the ear in place for intravital imaging, which will be done over a period of no more than one hour. **Depth of anesthesia will be frequently monitored by signs of spontaneous movement and whisker movement; foot pinch is of course not permitted as it will disrupt the live imaging, as will any animal spontaneous movement.** We have done pilot studies on ears from euthanized mice and found that we can image through the ear to the level of the cartilage. The infectious agent will remain in the subcutaneous implant for the duration of the study, including imaging procedures, so there will be no direct exposure of the pathogen to the environment, workers, or microscopy equipment.

Using this method, mice may be used for serial imaging over several days, since no further invasive procedures are done to the mice after initial implantation. **After each imaging**

session, recovery from anesthesia will be monitored continuously for the next 30-60 minutes until the mouse is spontaneously moving around the cage.

Following completion of the study at time points up to 8 days post-implantation, **mice will be humanely euthanized by cervical dislocation under anesthesia, and tissues will be collected.**

Pain and Distress Category - 2

Study Populations:

For this study, we anticipate using a variety of transgenic reporter mice, with up to 50 mice per year.

Risks to personnel:

The *Staphylococcus aureus* strain UAMS-1 is a non-MRSA clinical isolate that will in some cases harbor a plasmid expressing the fluorescent protein EGFP; we will not be using any MRSA strains in live infection models here. This bacteria is a clinical isolate and can infect humans, but it can be handled under conventional BSL2 protocols, and the microscope objective and other equipment will be disinfected using 70% ethanol. Any potentially contaminated surfaces will also be cleaned using 70% ethanol, or 10% bleach.

All procedures will be performed in accordance with the IBC approved Biosafety SOP. Prior to initiating activities with Staphylococcus aureus:

1. The PI and lab members will meet with the Biosafety Officer, Campus Veterinarian and vivaria staff to review vivaria safety practices.
2. Lab members will review the procedures with the Biosafety Officer using a fluorescent indicator (e.g. germ glo) to confirm the efficacy of safety precautions.

Committee Use Only

Final Disposition of this protocol:

_____ Approved by Chair/Vice-Chair

____XX____ Approved by IACUC Review

_____ Not Approved by IACUC Review

_____ Withdrawn by Investigator

Date of Action: ___6___/__29__/__2016____