

Immunization to Determine Immune Status and Antibody Production in Mice
IACUC Standard Procedure
Effective Date: May 2016



Description of procedure:

Mice are injected intraperitoneally or subcutaneously once with protein or peptide antigens in adjuvant. Typically, the adjuvant will be alum (aluminum potassium sulfate) and the antigen will be Trinitrophenol (TNP)-conjugated to keyhole limpet hemocyanin (KLH), although other protein antigens may be used (such as the recombinant Leishmania LACK antigen, pigeon cytochrome C, or ovalbumin) and other adjuvants may also be used (e.g. complete Freund's adjuvant - CFA). Mice are injected IP or SC with the antigen/adjuvant precipitate (1-100 ug protein in 5-10% alum or other suitable adjuvant) in 100-200 mcl sterile saline. If given subcutaneously, no more than 0.05 mls will be injected per site.

The purpose of this procedure is to monitor antibody production in response to the foreign antigens. 50-100 mcl of blood will be collected at various time points per UCSF blood collection guidelines as specified in the protocol (sub-mandibular blood collection is preferred) to establish the change in antibody titer. The mice are typically euthanized at the end of the 14-day period per the UCSF Policy on Euthanasia for Rodent Species, however they may be kept alive for a period of 2-6 months to study long-term immunity. In this situation, animals are re-immunized after 4-6 weeks and then bled several days after the booster immunization to monitor secondary or memory antibody response. This re-immunization is performed using the same antigen preparation as was used initially, unless the primary adjuvant was complete Freund's adjuvant, in which case the secondary immunization will be performed with incomplete Freund's adjuvant or without adjuvant.

The protocol must identify:

- The antigen and adjuvant to be used
- The time points for blood collection
- If secondary immunization will be performed
- The endpoint of the experiment

Agents:

This procedure requires antigen, adjuvant and isoflurane. All agents administered to animals should be listed in the "Agents" section of the RIO IACUC protocol.

Adverse Effects:

Adverse effects should be listed in the "Adverse Effects" section of the RIO IACUC protocol.

Examples of potential adverse effects include: Skin irritation or ulceration