Protocol

Lymphocyte Deficiency Induced by Sublethal Irradiation in *Xenopus*

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In many studies of diseases affecting amphibians, it is important to determine to what extent lymphocyte-mediated defenses are involved. For example, in studies of the nature of the immune response of *Xenopus laevis* to the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, it was essential to determine if mucosal antimicrobial peptides or lymphocyte-mediated immunity was most important for resistance to this skin pathogen. In this protocol, we describe a method for sublethal irradiation to reduce lymphocyte numbers. Briefly, *X. laevis* adults or tadpoles are exposed to 9 Gy (900 rads) of irradiation applied by exposure to a cesium source or gamma irradiator to reduce lymphocyte populations in the spleen.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

Dechlorinated tap water for frogs (filter-sterilized)
Penicillin stock solution (10,000 IU/mL)
Streptomycin stock solution (10,000 µg/mL)
Xenopus laevis adults (small; 4–5 g in weight) or tadpoles

Equipment

Clean opaque plastic containers (12 cm × 8.5 cm × 5 cm) with vented lids to accommodate small *Xenopus laevis* adults (4–5 g in weight) or tadpoles
Sterile, plastic 500-mL container to dilute antibiotics
X-ray source (alternative sources such as a Cesium-137 source or a Cobalt-60 source could be substituted)

For studies at Vanderbilt, the X-ray source was a 300 kVp/10 mA tube manufactured by Pantak (East Haven, Connecticut, USA). The half value layer was 0.73 mm Cu. In Rochester, we are now using a Gammacell 40 Exactor Low Dose device that uses Cesium-137 sources and is designed for low dose irradiations of small animals such as mice.

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METHOD

1. Place small *X. laevis* frogs at ~1–2 yr of age (4–5 g) individually in plastic containers in a small volume of dechlorinated tap water (~25 mL) to keep the frogs moist. Cover the vented containers with a lid to prevent the frogs from escaping. If using tadpoles, place them individually in similar containers with less water (10 mL).
2. Expose the frogs or tadpoles to the irradiation source for a sufficient time to absorb 9 Gy (900 rads) of irradiation.

   In the Ramsey et al. (2010) study, the frogs in their containers were placed 80 cm from the source, and the dose was applied at a rate of 0.8 Gy/min for exactly 11.25 min.
3. At the end of the irradiation period, replace the original water with a small volume of fresh dechlorinated water sufficient to completely immerse the frogs (~150 mL). Dilute stock solution antibiotics such that the water contains 0.005 IU of penicillin and 0.005 µg of streptomycin as a precaution to limit possible bacterial infections due to the irradiation.

   See Troubleshooting.

TROUBLESHOOTING

Problem (Step 3): Because the treatment compromises the immune system, any asymptomatic infection may be revealed.

Solution: It is recommended to include a control group that is only sublethally irradiated and not undergoing any other treatment. Notably, when we investigated adaptive immunity against ranavirus pathogens, several of the controls that had only been irradiated (not infected experimentally) died from ranaviral infection, which led to the discovery of persistent asymptomatic ranaviral infections in a significant fraction of *X. laevis* obtained from different suppliers (Robert et al. 2007).

DISCUSSION

Xenopus laevis is a species frequently used as a model for studies of amphibian immunity to disease. There is a rich literature describing the complexity of the amphibian immune system, based largely on studies using *X. laevis*. (See Robert and Ohta 2009; Flajnik 2018 for recent reviews.)

Compared to mice for which 10 Gy is a lethal dose, *X. laevis* adults and tadpoles are relatively more resistant to radiation and tolerate 9 Gy of irradiation without significant mortality. The dose of sublethal irradiation recommended here is sufficient to reduce spleen cell numbers (Ramsey et al. 2010), impair tumor rejection (Rau et al. 2001), impair the ability to resist infection and reemergence of disease caused by ranaviruses (Robert et al. 2005, 2007), and impair the ability to resist infection caused by *B. dendrobatidis* (Ramsey et al. 2010). Analysis of the spleens of irradiated frogs showed that the numbers of leukocytes were markedly reduced by the irradiation (Ramsey et al. 2010). Irradiated frogs maintained their weight for greater than 45 d, suggesting that the irradiation did not impair the functions of the gastrointestinal tract (Ramsey et al. 2010). Irradiated frogs showed no evidence of skin damage due to the procedure (Rau et al. 2001; Ramsey et al. 2010). It is, however, possible to use a lower irradiation dose (6 Gy) to further minimize side effects on skin and intestine epithelia. These lower doses have been found to be effective for depletion of thymocytes in *X. laevis* adults and tadpoles, but they did not markedly affect the thymic stroma, a more radio-resistant compartment (Goyos et al. 2009). Similarly, a 10 Gy dose was also effective to inhibit survival of thymocytes of *X. tropicalis* (Goyos et al. 2011) and would likely be applicable to other nonmodel amphibians to help characterize adaptive immune responses to pathogens.

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