UCSF Office of Research
Institutional Animal
Care and Use Program

Production of Hematopoietic Chimeras

IACUC Standard Procedure
Effective Date: August 2022

Description of procedure:
Bone marrow or fetal liver cells of a desired mouse strain are isolated and collected from euthanized donor mice. These will be delivered alone or mixed at a 1:1, 1:10, or other desired ratio, with bone marrow or fetal liver cells from another mouse strain of interest and are then transferred into lethally irradiated recipient mice.

Irradiation of recipient mice is performed by using an irradiator within one of the barrier facilities. Mice are placed into a rotating pie-shaped holder (to limit mobility and ensure equal irradiation), which is then secured in the irradiator to deliver a dose of 350R (sublethal) - 600R (lethal). The optimal dose may vary per strain[1-7]. To accomplish complete myeloablation, use the lethal dose (e.g. 600R), which may be fractionated into two doses (e.g., repeated within 3 hours). In the protocol Section I, list the dose to be administered and the interval between doses if splitting. Split dose irradiation is used to limit the non-hematopoietic toxicity, usually intestinal damage. After 2-3 days, mice with lethal irradiation may experience acute illness (e.g., if transplant does not engraft properly) and should be monitored for this potential adverse effect.

Irradiated mice are injected within 18h after the second irradiation via the tail vein or retro-orbitally as described in your protocol.

Each irradiated mouse will receive a single injection of donor bone marrow cells. Review the standard procedures for Tail Vein Injection and Retro Orbital Injection. While usually not required when using irradiated wild-type mice as recipients, when using irradiated genetically immune compromised (e.g. RAG-deficient) mice as recipients it is recommended to maintain them on antibiotic-containing water or feed for approximately 4 weeks. This is advised even for sublethal dose of irradiation. Duration of the entire procedure is typically 6-12 weeks (*deviations to be noted in approved IACUC protocol*).

Agents:
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: transplant failure, anemia, infection, intestinal bleeding

References

1. Kallman RF, Kohn HI. The influence of strain on acute x-ray lethality in the mouse. I. LD50 and death rate studies. **Radiat Res. 1956 Oct;5(4):309-17**.
2. Grahn D, Hamilton KF. Genetic Variation in the Acute Lethal Response of Four Inbred Mouse Strains to Whole Body X-Irradiation. **Genetics. 1957 May;42(3):189-98**.

3. Grahn D. Acute Radiation Response of Mice from a Cross between Radiosensitive and Radioresistant Strains. **Genetics. 1958 Sep;43(5):835-43**.
4. Mori N, Okumoto M, Morimoto J, Imai S, Matsuyama T, Takamori Y, Yagasaki O. Genetic analysis of susceptibility to radiation-induced apoptosis of thymocytes in mice. **Int J Radiat Biol. 1992 Aug;62(2):153-9**.
5. Iwakawa M, Noda S, Ohta T, Ohira C, Lee R, Goto M, Wakabayashi M, Matsui Y, Harada Y, Imai T. Different radiation susceptibility among five strains of mice detected by a skin reaction. **J Radiat Res. 2003 Mar;44(1):7-13**.
6. Ohta T, Iwakawa M, Oohira C, Noda S, Minfu Y, Goto M, Tanaka H, Harada Y, Imai T. Fractionated irradiation augments inter-strain variation of skin reactions among three strains of mice. **J Radiat Res. 2004 Dec;45(4):515-9**.
7. Biedermann KA, Sun JR, Giaccia AJ, Tosto LM, Brown JM. scid mutation in mice confers hypersensitivity to ionizing radiation and a deficiency in DNA double-strand break repair. **Proc Natl Acad Sci U S A. 1991 Feb 15;88(4):1394-7**.