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Generation of Bone Marrow and Fetal Liver Chimeric Mice

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Abstract

The creation of bone marrow and fetal liver chimeric mice has proven to be a valuable tool in the field of immunology. Chimeric mice are used to study the contribution of various cell types of hematopoietic versus non-hematopoietic origin in the course of an immune response. In this chapter, we describe a detailed method to obtain bone marrow or fetal liver chimeric mice and assess the efficiency of donor cells to repopulate the hematopoietic compartment of recipient mice.

Keywords

Bone marrow transplant; Fetal liver transplant; Congenically labeled cells; Radiation; Assessment of reconstitution; Antibodies

1 Introduction

Transplantation of congenically labeled bone marrow or fetal liver cells into lethally irradiated mice has significantly improved our ability to study cellular and humoral immune responses. Many studies use this technique to distinguish the contribution of hematopoietic cells versus non-hematopoietic cells during the course of an immune response [1, 2]. A variety of immune responses can be recreated in vitro using cell lines and in vitro-differentiated cells (dendritic cells, macrophages, T and B cells) [3–5]. However, these studies are limited due to the complexity and time-course of many immune reactions that take place in vivo. Bone marrow chimeras have allowed the study of hematopoietic cell development and their participation in long-term immune responses in a physiological setting.

Reconstitution of lethally irradiated mice can be conducted using cells obtained from (a) bone marrow of adult donor mice [6] and (b) fetal liver cells obtained from embryonic day 14 (E14) mouse fetuses [7]. Reconstitution of lethally irradiated mice with fetal liver cells is useful when studying the immune system of embryonic lethal animals. Here, we describe how to prepare radiation chimeras using both sources of hematopoietic cells. This protocol utilizes congenic mouse strains that differ at the common leukocyte antigen (CD45) locus. The CD45 antigen is expressed by all nucleated cells and allows donor cells to be easily distinguished from host cells [8]. Expression of the CD45 allele on the surface of nucleated cells has proven to be advantageous not only for detection of donor cells, but also for their isolation and usage in subsequent assays.

Recipient mice are subjected to predetermined lethal or sublethal doses of X-ray or γ radiation and then injected with donor bone marrow- or fetal liver-derived cells. The mice are then screened by flow cytometry for the presence of donor cells 4–6 weeks post adoptive transfer. A detailed study published in 1988 by Spangrude et al. demonstrates the ability of

hematopoietic precursor cells to reconstitute all blood cell types [9]. Additionally, the long-term fate of reconstituted hematopoietic cells has been followed and published by Jordan and Lemischka in 1990 [10]. This chapter aims to provide a comprehensive method by which to obtain chimeric mice and notes common measures that can be taken to ensure successful transplantation and mouse survival.

2 Materials

2.1 Preparation of Bone Marrow or Fetal Liver Chimeras

1. Bone marrow recipient animals: C57BL/6 males, 8–10 weeks old (National Cancer Institute).
2. Bone marrow donor animals: C57BL/6 CD45.1 males, 2–5 months old (National Cancer Institute).
3. Fetal liver donor animals: C57BL/6 CD45.1 animals, embryonic day 14.
4. Control bone marrow donor animal: C57BL/6 CD45.2 males, 2–5 months old (National Cancer Institute).
5. X-ray machine or cesium irradiator for γ irradiation.
6. Mouse irradiation chambers (Braintree Scientific, Inc.).
7. Animal CO_2 euthanasia chamber.
8. Antibiotic water: 2 mg/ml of neomycin sulfate prepared in autoclaved water, pH = 2 with 2 N HCl.
9. Cold phosphate-buffered saline (1× PBS).
10. PBS with 3 % fetal bovine serum (FBS).
11. Straight surgical forceps and scissors.
12. Non-tissue culture-treated sterile petri dishes (100 × 20 mm).
13. 70 % Ethanol.
14. 1 ml syringes with 27-guage needles.
15. Cell strainers (70 μm).
16. Mouse restraining chamber (Braintree Scientific, Inc.).
17. Infrared heating lamp.

2.2 Assessment of Reconstitution

1. Irradiated and reconstituted mice.
2. Heparin tubes for blood collection (Becton Dickinson).
3. Submandibular bleeding lancets.
4. PBS.
5. Red cell lysis buffer (Beckman Coulter).
6. PBS containing 3 % FBS.
7. FACS buffer: PBS, 3 % FBS, 0.04 % Sodium Azide. Mix, filter sterilize, and store at 4 °C.

8. Monoclonal antibodies to detect recipient and donor hematopoietic cells (CD45.1 and CD45.2 alleles), as well as B cells and T cells (CD19, CD3, CD4, and CD8) (Beckman Coulter).
9. 5 ml centrifuge tubes or 96-well plate.
10. Centrifuge.
11. Flow cytometer.

3 Methods

3.1 Preparation of Bone Marrow Chimeras

1. Irradiate bone marrow-recipient animals using the X-ray machine or the cesium irradiator at the appropriate dose as detailed below.
2. The animals are placed in a pie chamber (holds up to 11 mice). This method allows for even delivery of the radiation dose by ensuring that all animals are placed at the same distance from the radiation source. It is important to reduce the amount of time the animals will spend in the pie chamber to maintain sufficient oxygen flow and reduce overheating of the animals.
3. The irradiation dose will vary by mouse strain. The laboratory should determine the appropriate dose of irradiation for their mouse strain. This protocol will outline the doses used for C57BL/6 wild-type animals.
4. Animals are exposed to a lethal dose of irradiation (900–1,100 rad) to prevent recovery of endogenous hematopoietic cells. Radiation can be delivered in one single dose the day before bone marrow transplant or in two equal doses 6 h apart to minimize damage of gut and lung cells. If the radiation dose is split into two equal doses the animals should be exposed to the highest dose of irradiation (1,100 rad) to achieve elimination of endogenous progenitor cells (*see* Note 1).
5. Euthanize the donor mice: C57BL/6 CD45.1 males and one control C57BL/6 male as approved by the institution's animal protocol.
6. Isolate bone marrow cells using the following protocol: Spray the mice with 70 % ethanol and cut the abdominal skin. Cut the skin all the way to the hind legs and remove it using forceps and scissors. Remove the feet and place the legs in 70 % ethanol for 2 min. Transfer the legs to cold PBS for 2 min, two times. Remove excess flesh from the leg bone and cut off the ends of the bones. Place the bones in media while flushing the bone marrow. Flush the bones using a 27-guage needle.
7. Once finished, make a single-cell suspension of all the marrow and passage the cells through a strainer.
8. Pool cells from all mice and adjust the density to 5×10^6 cells/ml of PBS. One mouse yields about 70×10^6 bone marrow cells. In the author's laboratory, cells are usually pooled from three different mice to ensure sufficient number of cells to perform bone marrow transplants into a large number of animals.
9. Inject 200 μl of bone marrow cell suspension intravenously into irradiated recipient and control animals (*see* Note 2). One can inject anywhere from 1 to 5×10^6 cells/recipient.

[1]T cells are the most radiation-resistant cell type. Therefore, if the irradiation dose will be split into two separate doses it is recommended that at least a combined dose of 1,100 rad be given to the animals. Irradiate the mice the day before bone marrow transplant.

10. Maintain animals on neomycin sulfate water (2 mg/ml) for 2 weeks following transplantation to prevent infection. Fill a 2 L glass bottle with water and adjust the pH using HCl. Autoclave sterilize water and allow cooling to room temperature. Water is autoclave sterilized to ensure removal of *Pseudomonas aeruginosa*, a common waterborne pathogen. Add 4 g of neomycin sulfate and allow it to dissolve. Wrap the bottle with aluminum foil to protect the water from light. Water can be stored at 4 °C for up to a week. We replace the water in the mouse cages two times a week.
11. Six to eight weeks post transplantation, recipient mice can be analyzed for the presence of donor-derived cells via flow cytometry.

3.2 Preparation of Fetal Liver Chimeras

1. Irradiate the fetal liver-recipient animals using the X-ray machine or the cesium irradiator at the appropriate dose as detailed in Subheading 3.1.
2. Euthanize the donor mice: C57BL/6 CD45.1 pregnant female mice at gestation day 14 (*see* Note 3).
3. Isolate fetal livers using the following protocol: Spray the mice with 70 % ethanol and cut the abdominal skin. Expose the embryonic sac. Using forceps and scissors remove the embryos and place in a petri dish with media (the embryos will look like pearls on a string). Remove individual embryos from embryonic sac and remove the surrounding tissue. Cut the abdominal skin of the embryos and expose the fetal liver. The fetal liver is very fragile and should be carefully removed using two forceps.
4. Once finished, make a single-cell suspension of the fetal liver cells by passing the liver through a 1 ml pipette tip. Passage the cells through a strainer.
5. Pool cells from all mice and adjust the density to 5×10^6 cells/ml of PBS. One mouse yields about 5×10^6 fetal liver cells.
6. Inject 200 μl of the fetal liver cell suspension intravenously into irradiated recipient and control animals. One can inject anywhere from 1 to 5×10^6 cells/recipient.
7. Maintain animals on neomycin sulfate water (2 mg/ml) for 2 weeks following transplantation to prevent infection.
8. Six to eight weeks post transplantation, recipient mice can be analyzed for the presence of donor-derived cells via flow cytometry.

3.3 Screening Animals for Donor-Derived Cells

1. Collect 200 μl of peripheral blood from irradiated and reconstituted animals into heparinated tubes to prevent clotting. Resuspend the blood in heparin by inverting the tubes 2–3 times.
2. Add 100 μl of blood into 2 ml of red blood cell lysis buffer and resuspend by vortexing. The solution will go from turbid to clear red as the cells lyse (2–3 min).

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[2]We find that it is easier to perform tail vain injections with mice whose body temperature is brought closer to 38 °C. We usually use an infrared heat lamp to increase the body temperature of the animals. The increase in temperature exposes the veins to the surface for injections.

[3]In order to obtain E14 embryos, female and male mice are bred overnight. Male mice are taken out of the cage the following morning and females are checked for the presence of plugs. Fourteen days post breeding, pregnant mice are euthanized and the E14 pups are harvested. Fetal livers are harvested from the embryos and resuspended into single cells. If genotyping of the embryos is required at the time of harvest, the fetal livers can be placed on ice for several hours. An expedited genotyping protocol is recommended in order to minimize the number of dying cells.

Add 2 ml of 3 % FBS/PBS to all tubes and spin down to pellet cells. Wash all cells one more time and aspirate the solution.

3. Resuspend the cell pellet in FACS buffer (*see* Note 4). Transfer cells into several 5-ml centrifuge tubes or a 96-well plate depending on the number of stains that will be evaluated.
4. Prepare the antibody cocktail for staining. Make a master mix for all of the stains (*see* Note 5).
5. The cells will be stained with anti-CD45.1 and CD45.2 antibodies to determine the donor vs. host cell composition. Often T cell markers, such as CD3, CD4, and CD8, are added to the staining cocktail to determine the percentage of donor T cells. T lymphocytes are the most radiation-resistant cell type and usually take the longest to be reconstituted [11].

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[4]Resuspend cells at 20×10^6 cells per mL of FACS buffer. We use 50 μl for each stain that will be transplanted. If screening a large number of mice, a 96-well plate is recommended for staining as it saves time during staining and washing.
[5]Titrations should be performed for all antibodies prior to use to identify the proper concentration for optimal staining. A master mix for all stains is also recommended to limit sample-to-sample variability.

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