NEONATAL TRANSPLANTATION OF HEMATOPOIETIC CELLS

Preconditioning / Myeloablation Procedure

1. Remove the dam (mouse mom) from the cage into a clean cage, transfer pups into the irradiator dish, irradiate with dosing listed below, pups go back into the cage, sprinkle with bedding, then mom goes back in to join them.

About 3 hours before transplantation -
NSG neonates receive 100 rads (cGy).
Rag2-/- Common Gamma-/- neonates receive 250 rads (cGy).
C57BL/6 and B6.SJL (BoyJ) receive 300 rads (cGy).

Prepare cells

2. Run cells through a 70 um strainer.

3. Resuspend cells in PBS volume sufficient for 15 ul per pup. You should know how many pups you have because you've already irradiated them. You should add a dose for an additional pup because volume can be "lost" in the syringe or during handling.

Try to transplant between 50,000 to 1-2 million cells per neonate, depending upon the cell source and frequency of engraftable cells in the cell suspension. Trace amounts of serum are ok, but avoid resuspending in PBS containing serum, as this could cause inflammatory response.

Transplant neonates

4. Remove the mom from the cage and place into a clean cage. Remove her from the area so she doesn't know that you're handling her pups. This includes sight, smell, and sound.

5. Place one or two pups on ice. Three is ok when you're quite proficient. Carefully observe them for color and movement. They are not ready for injection if they are moving. They should look slightly purple and be nonresponsive to handling or toe pinch. The facial vein is easiest to target when purple in color.

If you encounter delays, transfer pups to heat and start over after several minutes. Over-chilled pups may be difficult to inject due to vessel constriction and may not revive. Each pup can tolerate cooling two times, if necessary. If pup has been on ice over 15 minutes, transfer to heat.

6. Inject 15 ul volume via facial vein with a Hamilton syringe and 30-gauge needle. Two aspects are critical during this procedure: 1. positioning of the pup, 2. minimal movement of the hand while pushing the plunger. Beveled edge should face upward away from the pup. You must secure the skin of the head to resist the needle as it enters the vein. If skin is stretched too tightly, the vein will "disappear" and you must loosen your grip.

If the needle is well placed in the vein, the vein will clear (red disappears) as cell suspension is injected. If the needle misses the vein, a fluid bubble will form as you inject. We have observed engraftment even when the vein is missed.

7. Transfer pups onto gentle heat. Cold stainless steel under the pups should be insulated by a heating pad (on LOW setting) or other material that can absorb heat from the heat lamp. Pups will regain pink tone and be very mobile. Be careful that they don't wiggle away into a dangerous area.

8. Accumulate all the pups in the recovery area under the heat lamp, then transfer all together back into the cage. Sprinkle them with bedding to mask any odors from your gloves, then move the mom back in to join them.

Extra Tips & Comments

We generally set up the male and female(s) that are to provide the neonates 3 weeks exactly from the day we plan to transplant. In other words, pair the mice on WED, check plugs THURS, then transplant cells 3 weeks later on WED. It's easiest to transplant with a Hamilton syringe and 30-gauge needle the day after delivery. As the pups get older, it's more challenging. We temporarily remove the mom from the cage into a clean cage, transfer pups into

the irradiator, then pups go back into the cage, sprinkle with bedding, then mom goes back in to join them. The same procedure is used a few hours later when you inject the cells; you don't want the mom to know that you're handling her pups. Warming the pups after immobilizing/anesthetizing on ice is also critical. They shouldn't scorch under a heat lamp, but we try to warm gently from above and below.

Modified by Pamela L. Wenzel, 2/21/2013