Humanization of NSG/NOG mice

I. Transplantation of human CD34+ hematopoietic stem cells

Mice: newborn NOG/NSG pups are used

Procedure

1. Condition newborn pups before cell transplantation,
2. Separate dam from pups into a separate cage
3. Irradiate pups in nest within the cage at 1 Gy using a Rad Source R2000 X-ray irradiator (55 second at indicated level).
4. Return dam back to the nest.
   Note 1: *Cage should be observed to ensure that dam does take care of pups.*
   Note 2: *Human CD34+ hematopoietic stem cells should be transplanted into pups between four to 24 hours after irradiation.*
   Note 3: *Ranging from two to seven littermates are reconstituted with one cord blood sample derived from one donor.*
5. Resuspend CD34+ HSC cells in PBS at a concentration of $5x10^4$ cells /30 µl PBS in total volume corresponding to the litter size.
   Note 4: *For two pups 60 µl cells needed; for seven pups 280 µl cells needed*
6. Separate dam from pups by placing in clean cage
7. Inject newborn pups intrahepatically (*i.h.*) using a 30-gauge needle insulin syringe. Each pup is transplanted with $5x10^4$ cells in 30 µl volume.
8. Returned dam to the nest.
9. Wean humanized animals at 28 days of age
   Note 5: *Due to irradiation at birth humanized mice are smaller in size and later weaning will reduce animal loss.*
10. When mice reach 3 months of age, the quality of reconstitution is analyzed by determining human immune cells amounts in peripheral blood as described in Part II.

II. Flow cytometry of mouse peripheral blood.

For the characterization of mouse repopulation by human immune cells use a basic 6-color panel of antibodies. Use human pan-CD45, CD3, CD4, CD8, CD14, and CD19 markers for a basic assessment.

Procedure

1. Peripheral blood samples are collected from the submandibular vein by using lancets (MEDIpoint, Inc., Mineola, NY) in EDTA-coated tubes.
2. Collect 100-µL aliquots of whole blood are
3. Centrifuge at 2000 *g* for 8 min to separate plasma from blood cells.
4. Remove plasma and add equal amounts of BSA/2% BSA to pelleted cells
5. Resuspend blood cells by gentle mixing.
6. Incubate blood cells with respective antibodies for 30 minutes at 4°C.
7. Remove red blood cells with FACS Lysing Solution (Becton Dickinson, San Jose, CA)
8. Wash cells twice with PBS containing 2% fetal bovine serum.
9. Test blood leukocytes for human pan-CD45, CD3, CD4, CD8, CD14, and CD19 markers using a multicolor panel.

Note 6: *Antibodies and isotype controls are obtained from BD Pharmingen (San Diego, CA),*

10. Analyze staining with a FACS LSR II (BD Immunocytometry Systems, Mountain View, CA) or Attune™ or other available instrument in the UNMC imaging core facility.
    Note 7: *Pre-test all lots of antibodies for optimal results.*
11. The results are expressed as percentages of the total number of gated lymphocytes. The gating strategy is human CD45⇒CD3⇒CD4/CD8, CD45⇒CD19, and CD45⇒CD14. Representative plots are shown on **Figure 1**.
12. Results we express as percentages of total numbers of gated lymphocytes and absolute number of cells per microliter of blood.

Note 8: *By three months of age, over 80% of animals are suitable for experiments. Non-reconstituted mice are euthanized.*

Note 9: *The animals with an absolute count of human CD45 > 800 cells/μL and CD3 >100 cells/μL are analyzed at four months of age. Animals that showed stable reconstitution with an absolute number of human CD45+ cells >2000/μL are selected for experiments.*

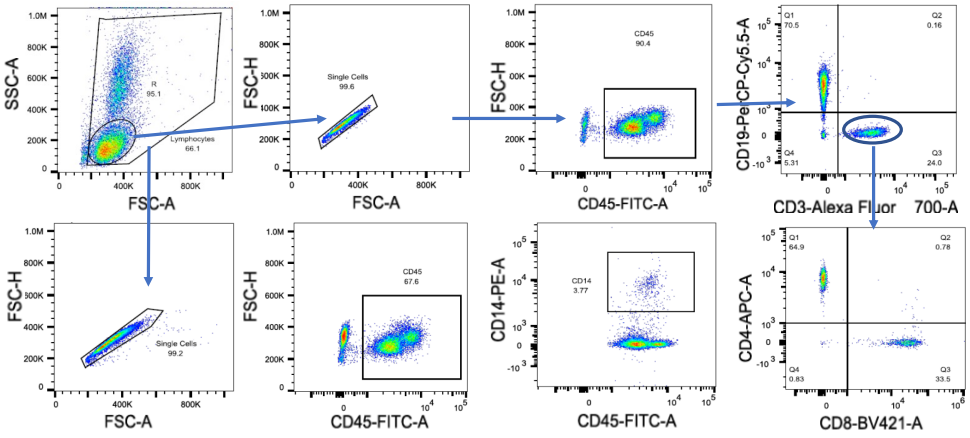

Figure 1. Example of flow cytometry analysis of humanized mouse peripheral blood.