**Description of Procedures:** All of the proposed experiments use mice from \_\_\_\_\_and \_\_\_\_\_\_ days old. All of the mice will be bred and housed in our mouse vivarium and will either be purchased from approved vendors or provided by our collaborators who are also at IACUC certified institutions. We typically use \_\_\_\_\_\_ mice per group for these experiments. Statistical power analyses have shown that given the size and variance of the immunologic and SLE outcome measures. Measurements, \_\_\_\_\_\_ mice per group gives a 85% chance of finding statistically significant (p < 0.05) differences.

More specifically, humanized mice from the CCHMC Xenograft Core to induce lupus like conditions by injecting blood from a lupus nephritis patient (IRB# 2013-1950) with a healthy donor as a control. Urine will be collected from these Lupus engrafted mice to measure protein. Most SLE mice die in 5-8 weeks post engraftment of 10 million PBNCs from lupus nephritis patient while mice receiving healthy donor cells live without obvious symptoms for more than 6 months. All these observations reflect the lupus like manifestation in NSG mice. We will also engraft NSG mice with ~8x106 PBMCs. We will have 5 different groups of PBMC donors: (i) with active SLE, no mutations in *FCGR2B*; (ii) active SLE, mutated *FCGR2B*; (iii) active SLE with underexpressed *FCGR2B*; (iv) healthy individuals and (v) non-engrafted mice. Briefly, freshly isolated by Ficoll-Paque Plus PBMCs from each donor will be injected intravenously in the tail vein of eight NSG mice. 4 weeks post injection mice will be bled, efficiency of the engraftment and titer of human IgG will be evaluated. Mice will be split in groups with equal efficiency of the engraftment for the subsequent treatment. Antibodies will be introduced via intra-peritoneal injection in amount of 0.2 mg/mouse 2 times per week for 2 weeks starting after 4 weeks of engraftment. On week six mice will be sacrificed; blood, spleen, and other tissues will be harvested and analyzed. In plasma we will evaluate total human IgG, IgG isotypes (eBioscience), anti-dsDNA (Zeus Scientific) and anti-cardiolipin (APL diagnostics) Abs.

**Justification:** In these experiments we will use laboratory mice as our model of choice. Mice, like humans, are \_\_\_\_\_\_\_\_\_\_ Hence, they have immune systems which closely parallel those of humans, thus enabling some generalizations to be made. Mice have been used in the great majority of previous experiments on the immune disorders. Consequently, there is a large existing data base and literature. Mice with specific immune defects, namely in our case the humanized mice from the CCHMC Xenograft Core and NSG mice, are particularly useful for delineating the physiology of an immune disorder that has potential immediate relevance to humans. Finally, laboratory-reared mice tend to respond rather similarly in terms of their immune responses various manipulations, such that the variance of the data can be kept low and the total number of required animals can be kept to a minimum

**Minimization of Paint and Distress:**

Drugs will be administered via with tail Vein Injection, Intramuscular Injection, Subcutaneous Injection, Intraperitoneal Injection, or Oral Gavage. Manual restraint or a restraint device will be used to minimize any trauma during these procedures.

In a normal rodent (non-obese) no more than 20% of body weight loss will be tolerated. Weight loss precedes animals becoming moribund. Thus, the loss of body weight is a sensitive indicator of ill-health. If any animal, engrafted or otherwise, loses 15% of body weight from peak weight, it will be closely monitored and vet staff alerted. The animal may be euthanized at this stage, if deemed appropriate. All animals loosing 20% bodyweight will be euthanized, irrespective.

Blood collection for serum/plasma cytokines, antibody titers, etc.

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| Collection of blood via the tail laceration/submandibular (i.e. glucose tolerance test) We will not exceed more than 7.7ml/KG/ Mouse in 2-weeks.  | 10 -100 µl from mice | Up to 6 times in 2 hours  | At least 5 min. |
| Submandibular vein | < 100 µl (mouse) | One | 1 week |
| Tail vein | < 30 µl (mouse) | 4 | 30 min |
| Orbital vein | ~ 100 µl (mouse) | 1 | once |

Method of Euthanasia:

Carbon Dioxide (compressed gas must be used) followed by either cervical dislocation.

**Veterinary care of animals:** The animal care and use program at the University of Cincinnati (UC) is centralized campus wide under Laboratory Animal Medical Services (LAMS). LAMS is staffed by three full time veterinarians, six veterinary technicians plus animal care staff. One LAMS veterinarian; one veterinary technician and one LAMS husbandry supervisor is on call after hours. All animals are cared for daily, 7 days per week. UC is fully accredited by AAALAC; the most recent site visit resulted in Continued Full Accreditation with no suggestions for improvement (date of site visit March 2-4, 2010; date of letter July 9, 2010). UC has an Animal Welfare Assurance on file with the NIH-OLAW, (Assurance Number A-3295-01, expires November 30,2015). UC fully complies with the Guide for the Care and Use of Laboratory Animals (Guide), the Public Health Service Policy on the Humane Care and Use of Laboratory Animals (PHS Policy) and all U.S. Animal Welfare

Act Regulations.