

**Drs. Megan Sykes and David Sachs**

This is a renewal of a highly synergistic PPG exploring tolerance induction for xenotransplantation. Themes include: 1) Tolerance of the adaptive immune system; 2) Overcoming innate immune barriers; and 3) Using advanced genetic engineering techniques to improve genetically modified (GM) MGH inbred miniature swine. In **Project 1**, “Achieving Xenograft Tolerance through Thymic Programming”, we will study and attempt to avoid the proteinuria which limits GalT-KO pig kidney transplant survival; we will optimize tolerance and protective immunity by transplanting a hybrid vascularized thymus (VT) containing pig and baboon TECs and combine this with mixed xenogeneic chimerism with intra-bone injection of pig bone marrow to simultaneously tolerize both adaptive and innate immunity. In **Project 2**, “Achieving Xenograft Tolerance through Mixed Chimerism”, we have achieved prolonged mixed chimerism in recipients of human CD47 (hCD47) Tg pig hematopoietic cells (HCs), resulting in remarkably prolonged pig skin graft survival; we will combine this approach with intrabone injection of hCD47 Tg/hCD55 Tg/GalT KO pig hematopoietic cells and use ex-vivo expanded recipient Tregs to enhance engraftment, aiming to achieve more durable chimerism and, with it, tolerance of B, NK and T cells; we will also test HCs from new GM swine produced in Project 4. **Project 3**, “Tolerance of Adaptive and Innate Human Anti-Pig Immune Responses in Humanized Mice”, combines mixed xenogeneic chimerism and porcine thymic transplantation in humanized mice with robust human immune systems; we will combine engineered pig/human hybrid thymi with mixed xenogeneic chimerism to achieve multi-faceted immune tolerance along with protective immunity for both human and porcine tissues; we will determine the impact of the hCD47 Tg on induction of porcine mixed chimerism in these mice and determine the impact of duration of this mixed chimerism on human T, B and NK cell tolerance. In **Project 4**, “Improving Xenogeneic Chimerism and Tolerance through Genome Engineering Technology”, advanced genetic engineering techniques will be used to generate two optimal GM pig lines, one for tolerance induction and the other for organ transplantation, on a background of inbred miniature swine with common GMs that will be needed for both purposes. **Core A** will provide administrative support for the PPG, facilitating interactions between the primary and subcontract sites. **Core B** will support all large animal needs; provide support for infectious diseases, coagulation and clotting issues; and provide mAbs and antisera. **The synergism of these projects and cores and the fertile interactions among them should bring us closer to the goal of clinical xenotransplantation.**