

Summary of T32 Research

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Project 1: Regulatory T Cell (Treg) Expansion

Xenotransplantation could help make up the difference in the numbers of organs needed for transplantation and the number available. Although we have achieved prolonged survival with good organ function in a pig-to-primate xenotransplantation model, we have not yet been able to eliminate the long-term effects of chronic antibody-mediated rejection. Mixed chimerism, mixing the donor immune system with the recipient immune system by bone marrow transplantation, reduces this problem in rodent models, but we have been unable to extend the success of this approach into the pig-to-primate model since we have thus far failed to suppress the B cell response. However, it has been shown that Tregs can suppress the B cell response, and recent models have been created for the ex vivo expansion of Tregs. Our goal was to develop a protocol for massive expansion of baboon Tregs for the purpose of infusion in a xenotransplantation model. We were able to expand baboon Tregs 100-fold over 1 month by stimulating them in culture with growth factors (such as IL-2) and antibodies directed against their key cell-surface activation receptors (such as the T cell receptor and the receptors for co-stimulatory signals). We then developed a method of cryopreserving and later thawing and reactivating these cells for future use so they could be banked in large numbers and used when required. Of note, this work was published earlier this year in *Transplantation Direct*. We have more recently turned our attention to growing Treg lines specific for the donor, which we hypothesize will provide better and more specific suppression.

Project 2: Long-term persistence of innate lymphoid cells in the gut after intestinal transplantation

Little is known about innate lymphoid cell (ILC) populations in the human gut, and the turnover of these cells and their subsets after transplantation has never been evaluated previously. It is thought that these cells play a role in defense against pathogens as well as the development of the remainder of the gut immune system. We compared the percentages of donor and recipient ILCs in the gut after isolated intestinal and multivisceral transplantation and found that donor ILCs persisted up to 8 years after transplantation, longer than other donor immune cells have been shown to persist. More specifically, cells expressing markers of the type of ILC that helps form the remainder of the immune system predominated among the donor-derived ILCs, and the specific type that most persisted is the type of ILC hypothesized to persist throughout life and give rise to other aspects of the gut immune system. We are currently in the process of submitting this work as a manuscript.

Project 3: Combined bone marrow plus liver transplantation in cynomolgus monkeys

Combined kidney/bone marrow transplantation (CKBMT) induces tolerance in both primates and humans. Since liver is thought to be more tolerogenic than kidney, we hypothesized that combined liver/bone marrow transplantation (CLBMT) should be successful. We performed this pre-clinical study in cynomolgus monkeys. Five pairs of animals were used. All were mismatched for class I, and the first 2 pairs were mismatched for class II. Liver and $\sim 3 \times 10^8$ donor bone marrow cells/kg were transplanted

on day 0. The induction regimen consisted of 1.5Gy total body irradiation as well as antibodies directed against T cells (ATGAM) and B cells (Rituximab) and 28-days of more traditional immunosuppression with calcineurin inhibitors such as cyclosporine or tacrolimus. In our first few animals, who experienced organ rejection after immunosuppression was stopped, we observed high percentages of CD8 T effector memory cells (TEM) in the blood, graft, and draining node but not peripheral nodes. Therefore, we added costimulatory blockade (anti-CD40) and depletion of CD2+ memory cells (LoCD2b) for future recipients. Subsequently this cell population did not expand. In contrast to the earlier recipients, recipients with the additional medications died of likely graft-versus-host disease and with no signs of rejection, even more than 30 days after all immunosuppression was stopped. We therefore conclude that CLBMT induces transient MC, but rejection eventually occurs. High levels of CD8 T effector memory cells likely contribute. However, when additional costimulatory blockade and CD2 depletion are given, these cells are significantly depleted, preventing rejection, prolonging MC, decreasing anti-donor cellular responses, and possibly permitting tolerance.

Project 4: Long-term outcomes of auxiliary partial orthotopic liver transplantation in pre-adolescent children with fulminant hepatic failure

By preserving part of the native liver, auxiliary partial orthotopic liver transplantation (APOLT) provides the advantage of potential immunosuppression withdrawal if the native liver recovers but has had limited acceptance, especially in the United States, due to technical complications and low rates of native liver regeneration. No previous study has evaluated APOLT specifically for pre-adolescent children with fulminant hepatic failure (FHF). This population might benefit especially based on greater capacity for liver regeneration. Data from 13 pre-adolescent children who underwent APOLT were compared to 13 matched controls who underwent orthotopic liver transplantation (OLT) for FHF from 1996-2013. There were no significant differences in patient demographics or survival between the two groups. However, all surviving OLT recipients (10/13) remain on immunosuppression, while all but one surviving APOLT recipient (12/13) showed native liver regeneration, and the first ten recipients (76.9%) are currently off immunosuppression with two additional patients currently weaning. In our experience, APOLT produced excellent survival and high rates of native liver regeneration in pre-adolescent children with FHF. This represents the largest series to date to report such outcomes. Liberating these children from life-long immunosuppression without the downside of increased surgical morbidity makes APOLT an attractive alternative. In conclusion, we therefore propose that, with the availability of technical expertise and with the technical modifications above, APOLT for FHF should be strongly considered for pre-teenage children with FHF, including by other American centers.

Project 5: HLA matching affects patient survival, graft survival, and incidence of rejection episodes in largest analysis to date

Though human leukocyte antigen (HLA) matching between donor and recipient affects both graft and patient survival after kidney and heart transplantation, prior analysis has failed to produce a definitive conclusion as to whether HLA matching has a similar effect after liver transplantation. This is the first study to address this question using the Scientific Registry of Transplant Recipients (SRTR) database. The study population consisted of all adult subjects in the SRTR database who received a liver graft between 2002-2012. Separate Cox proportional hazard models were constructed to analyze all-cause death and

all-cause graft failure censoring at 5 years of follow up. The association between mismatch and acute early rejection before hospital discharge was analyzed using logistic regression. Of 53,686 total patients, 41.2% had information about both donor and recipient HLA A, B, and DR types. Patients were grouped into those with 0 mismatches (0.42%), low (1-2) mismatches (4.38%), and high (3-6) mismatches (95.2%). There were no significant differences in patient or graft survival in univariate analysis. Controlling for age, gender, race, primary liver disease type, ABO blood type, MELD, donor type (living vs. deceased), and post-transplant immunosuppression, patients with 0 HLA mismatches had a higher risk of all-cause death compared to both patients with low HLA mismatches (HR=1.8, p=0.003) and high HLA mismatches (HR=1.71, p=0.005) and a higher risk of all-cause graft failure compared to patients with HLA mismatches (HR=1.8, p=0.032). Conversely, patients with high HLA mismatches had higher risk of early (during initial hospitalization) rejection episodes compared to those with low HLA mismatches (OR=1.85, p<0.001) with a strong trend towards higher incidence compared to those with 0 HLA mismatches (OR=4, p=0.053). In the largest analysis to date, and the only analysis to use SRTR data, we show that HLA matching affects both patient and graft survival and incidence of early rejection episodes. It is possible that the same HLA mismatch factors associated with early acute rejection episodes may also correlate with later graft failure or patient death. Exploring these possibilities, which include decreased tolerance or more graft-versus-host response with zero mismatches, will be the subject of future work.

Original Publications During This Period

1. **Weiner J**, Zuber J, Shonts B, Yang S, Martinez M, Farber D, Kato T, Sykes M. Long-term persistence of innate lymphoid cells in the gut after intestinal transplantation. (In preparation)
2. **Weiner J**, Cui Z, Mohan S, Verna E, Griesemer A, Emond JC. HLA matching affects patient survival, graft survival, and incidence of rejection episodes in largest analysis to date. (In preparation)
3. **Weiner J**, Griesemer A, Island E, Lobritto S, Martinez M, Selvaggi G, Lefkowitz J, Velasco M, Tryphonopoulos P, Emond JC, Tzakis A, Kato T. Long-term outcomes of auxiliary partial orthotopic liver transplantation in pre-adolescent children with fulminant hepatic failure. (Accepted: Liver Transplantation)
4. **Weiner J**, Duran-Struuck R, Zitsman J, Buhler L, Sondermeijer H, McMurchy A, Levings M, Sykes M, Griesemer A. Restimulation after cryopreservation and thawing preserves the phenotype and function of expanded baboon regulatory T cells. *Transplantation Direct*. 2015 Feb;1(1):1-7.
5. Scalea J, Vincenzo V, Gillon BC, **Weiner J**, Gianello P, Turcotte N, Arn JS, Yamada K, Sachs DH. Development of anti-donor antibody directed toward non-MHC antigens in tolerant animals. *Transplantation*. 2014 Sep 15;98(5):514-9.
6. **Weiner J**, Wu J, Martinez M, Lobritto S, Ovchinsky N, Rohde C, Griesemer A, Kato T. The use of bi-planar tissue expanders to augment abdominal domain in a pediatric intestinal transplant recipient. *Pediatr Transplant*. 2014 Aug;18(5):E174-9.

Oral Presentations During This Period

1. Alonso P, Zitsman J, Sondermeijer H, Woodland D, Kato Y, **Weiner J**, Griesemer A, Buhler L, McMurchy A, Levings M, Sykes M, Duran-Struuck R. Enhanced mixed hematopoietic chimerism and immune tolerance in monkeys after BMT across MHC barriers and infusion of polyclonal recipient Tregs. Federation of Clinical Immunology Societies, San Diego, California, June 2015.
2. Zuber J, Shonts B, Lau S-P, Coley S, Yang S, DeWolf S, **Weiner J**, Farber D, Shen Y, Martinez M, Kato T, Sykes M. Mixed chimerism and two-way alloimmune responses in human intestinal

- allografts. Federation of Clinical Immunology Societies, San Diego, California, June 2015.
3. Zuber J, Shonts B, Lau S-P, Coley S, Yang S, DeWolf S, **Weiner J**, Farber D, Shen Y, Martinez M, Kato T, Sykes M. Mixed chimerism and two-way alloimmune responses in human intestinal allografts. International Small Bowel Transplant Symposium, Buenos Aires, Argentina, Jun 2015.
 4. **Weiner J**, Duran-Struuck R, Zitsman J, Sykes M, Griesemer A. Resting and restimulation after cryopreservation restores regulatory T cell phenotype and function. 14th Annual John Jones Surgical Society Spring Meeting, June 2014.
 5. **Weiner J**, Duran-Struuck R, Zitsman J, Sykes M, Griesemer A. Resting and restimulation after cryopreservation restores regulatory T cell phenotype and function. The 23rd Annual Columbia Surgery Research Competition, May 2014. (First Prize)

Abstracts/Posters During This Period

1. **Weiner J**, Kato Y, Duran-Struuck R, Martinez M, Baker S, Alonso P, Zitsman J, Wu A, Houck P, Pinyavat T, Lefkowitz J, Chaudhry S, Kato T, Sykes M. Combined bone marrow plus liver transplantation in cynomolgus monkeys. Federation of Clinical Immunology Societies, San Diego, California, June 2015.
2. **Weiner J**, Kato Y, Duran-Struuck R, Martinez M, Baker S, Alonso P, Zitsman J, Wu A, Houck P, Pinyavat T, Lefkowitz J, Chaudhry S, Kato T, Sykes M. Combined bone marrow plus liver transplantation in cynomolgus monkeys. American Transplant Congress, Philadelphia, Pennsylvania, May 2015.
3. **Weiner J**, Cui Z, Mohan S, Verna E, Griesemer A, Emond JC. HLA matching affects patient survival, graft survival, and incidence of rejection episodes in largest analysis to date. American Transplant Congress, Philadelphia, Pennsylvania, May 2015.
4. **Weiner J**, Zuber J, Shonts B, Yang S, Martinez M, Kato T, Sykes M. Long-term persistence of innate lymphoid cells in the gut after intestinal transplantation. American Transplant Congress, Philadelphia, Pennsylvania, May 2015.
5. Zuber J, Shonts B, Lau SP, Coley S, Yang S, **Weiner J**, DeWolf S, Farber D, Shen Y, Martinez M, Kato T, Sykes M. Impact of Alloreactivity On Intra-Graft T Cell Homeostasis Following Small Bowel Transplantation. American Transplant Congress, Philadelphia, Pennsylvania, May 2015.
6. Alonso P, Zitsman J, Sondermeijer H, Woodland D, Kato Y, **Weiner J**, Griesemer A, Buhler L, McMurchy A, Levings M, Sykes M, Duran-Struuck R. Induction of durable mixed hematopoietic chimerism and immune tolerance in monkeys. American Society for Blood and Marrow Transplantation BMT Tandem Meetings, San Diego, California, February 2015.
7. **Weiner J**, Duran-Struuck R, Zitsman J, Sykes M, Griesemer A. Resting and restimulation after cryopreservation restores regulatory T cell phenotype and function. World Transplant Congress, San Francisco, California, July 2014.
8. **Weiner J**, Griesemer A, Island E, Lobritto S, Martinez M, Ovchinsky N, Selvaggi G, Lefkowitz J, Velasco M, Tryphonopolus P, Emond J, Tzakis A, Kato T. Long-term outcomes of auxiliary partial orthotopic liver transplantation in pre-teenage children with fulminant hepatic failure. World Transplant Congress, San Francisco, California, July 2014.
9. Kato Y, Griesemer A, Duran-Struuck R, Wu A, Woodland D, **Weiner J**, Ohmura Y, Martinez M, Alonso P, Baker S, Karolewski B, Lefkowitz J, Kawai T, Kato T, Sykes M. The first tolerance induction model for combined liver and donor bone marrow transplantation in cynomolgus monkey. World Transplant Congress, San Francisco, California, 2014.

Grants/Career Awards During This Period

1. Second Prize in 24th Annual Columbia Surgery Research Competition, May 2015
2. Columbia Department of Surgery Startup Grant 2014-2015
3. NIH T32 2013-2015
4. First Prize in 23rd Annual Columbia Surgery Research Competition, May 2014

5. Columbia Department of Surgery John Jones Fellowship 2013-2014: Awarded annually to the Columbia General Surgery Resident submitting the best research grant
6. Highest ranked grant in 2014-2015 Columbia Department of Surgery Startup Grant competition