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Clinical Research Course IRB protocol
Neutropenia and T-cell chimerism after solid organ trasplant

STUDY DESCRIPTION

1. Study Purpose and Rationale

Current strategies for liver and small bowel transplant generally necessitate lifelong immunosuppresion post-transplant in order to prevent graft rejection. While necessary to preserve graft survival, these immunosuppressive regimens contribute greatly to post-transplant morbidity and mortality: the most frequent cause of death after transplant is infection (1). Immunosuppression also increases the risk of certain types of cancers, and the medications can have serious side effects such as renal toxicity and encephalitis. Thus, achieving sustained immune tolerance of the grafted organ without the need for such pharmacological immunosuppression is a major goal in the field of organ transplantation (2). For example, there is currently an ongoing clinical trial aimed at slowly weaning immunsuppressive medications with close monitoring in certain patients (3).

A different approach to achieving immune tolerance for the allograft would be to replace the recipient's bone marrow with that of the donor, thereby creating a fully chimeric recipient, much like a bone marrow transplant used in the field of Hematology-Oncology (4). However, converting a patient to full bone marrow chimerism (containing hematopoietic cells completely form the donor) introduces risks that outweigh those associated with lifetime immunosuppressive drugs. Namely, these risks include the toxicity associated with the conditioning and eradication of recipient marrow, the high likelihood of graft failure, and most importantly, GVHD.

While achieving full chimerism would not be reasonable given the aforementioned risks, it has been shown that a state of *mixed* chimerism, in which the recipient has some autologous bone marrow and some bone marrow from the donor, can be achieved with much milder conditioning, followed by transfer of donor hematopoietic stem cells (HSCs) (5). There is precedence in the approach of achieving mixed chimerism to promote tolerance without developing GVHD, even across MHC barriers in mice (5). In monkeys, in kidney transplantation using mild conditioning, tolerance was achieved when mixed chimerism was induced, without GVHD (6). Studies in humans have shown similar results (7,8). It should be noted that the precise mechanisms by which mixed chimerism promotes tolerance without causing GVHD are incompletely understood and require additional study.

In the aforementioned examples involving kidney transplantation, conditioning, albeit non-myeloablative, was necessary to create space in the bone marrow for the donor HSCs. However, even mild conditioning regimens have toxicities, and a strategy that obviates the need for that would be useful. A possible alternative to be studied here takes into account that liver and small bowel contain a considerable amount of lymphoid tissue. These immune cells have the potential to mount a response against recipient bone marrow, a phenomenon referred to as a lymphohematopoeitc graft-versus-host reaction (LGVHR), thereby creating the niche necessary for the development of mixed chimeris. Such isolated LGHVR reactions have been found to occur without causing diffuse GVHD (4). This reaction would be expected to cause cytopenia, and many liver transplant patients (as many as

80%) become neutropenic at some point in the first few months post-transplant. The mechanism for neutropenia is not well delineated, and many potential factors have been suggested (medicines, viral infection, overall illness) One hypothesis to be tested here is that neutropenia reflects LGVHR against recipient bone marrow. If true, neutropenia should be associated with measureable donor T-cells in the peripheral blood, as a marker for their presence in the bone marrow. It may also follow that, if true, this state of neutropenia represent the presence of a niche in the bone marrow, presenting an opportunity to create durable mixed chimerism by the infustion of donor HSC.

A second hypothesis to be tested here is that chimerism identified in the peripheral blood post-transplant is associated with a reduced rate of rejection within the first 12 months post-transplant. Although the infusion of HSC may more reliably achieve mixed chimers, it has been shown that HSC present in donor liver can be found in recipient bone marrow after transplant. Therefore, it is possible that some degree of allograft tolerance may be associated with chimerism as identified in the peripheral blood (9).

2. Study Design and Statistical Procedures

This is a retrospective study in which patient clinical information (including serial complete blood counts (CBCs), medications, medical history, histopathology results) as well as stored samples of peripheral blood mononuclear cells (PBMCs), are available for review and analysis.

Subjects will be pooled from two cohorts of liver and/or small bowel transplant patients (including living and deceased liver donors): 77 subjects from Columbia University Medical Center (CUMC) whose samples are deposited in the BioBank Core at the Columbia Center for Translational Immunology, and an additional 234 subjects from an NIH sponsored consortium study, "A2ALL" (Adult to Adult Living Donor Liver Transplantation Cohort). Donor and recipient PBMCs have been frozen and stored, which will allow for HLA typing (a necessary step in determining chimerism) as well as recipient chimerism analysis at 1 week, 2 weeks, 1 month, 3 months, 6 months, and 9 months post-transplant.

Statistical procedures:

Chi squared analysis will be used to determine if there is a statistically significant difference in the presence of chimerism between the neutropenic and non-neutropenic subjects. Based on the total number of ~ 300 subjects and an estimated rate of chimerism in the non-neutropenic transplants of 5% (estimated based on published report in liver-kidney transplant of overall (neutropenic and non-neutropenic) chimerism of 10%) (10), this study should be able to show a statistically significant difference in chimerism between neutropenic and non-neutropenic subjects if neutropenic subjects' rate of chimerism is at least 15%, or three times that of the non-neutropenic subjects.

Chi squared analysis will also be used to determine if there is a statistically significant difference in the rate of rejection between patients with and without peripheral blood chimerism. Given the previous report of 10% chimerism overall in kidney transplant recipients (10) and given that on random sampling of patients from the CUMC cohort that ~65% of patients had some degree of rejection noted on biopsy within the first year post transplant, this study will be able to show a statistically significant difference in the rate of rejection between subjects with and without chimerism if the rejection rate for the patients with chimerism is < ~40%.

Logistic regression will be used to analyze possible covariates and confounders in the relationship between neutropenia and peripheral blood chimerism (e.g. medications, viral infection, age of recipient) as well as between peripheral blood chimerism and rejection (e.g. reason for transplant, age of recipient, age of donor).

3. Study Procedures

Absolute neutrophil count will be determined from complete blood count results already present in the electronic medical record. Absolute neutrophil count will be calculated in the standard way: ANC=white blood cells/mm^3 x (% neutrophils + % bands)/100. The standard definition of ANC of < 1500/mm^3 will be considered neutropenia.

Peripheral blood chimerism will be determined by flow cytometric analysis using combinations of fluorescently-labeled anti-HLA antibodies chosen to differentiate donor from recipient based on previously determined MHC typing. Donor-recipient pairs that have not yet been typed will need to be so beforehand. The presence of chimerism will be determined by the detection of donor cells in the recipient circulation above a certain threshold of detection of the flow cytometer and associated software.

Histopathology results present in the electronic medical record will be used to classify rejection in the first year after transplant. Subjects who have undergone liver biopsy after transplant and have shown any degree of rejection (Banff score 1-9; with rejection being scored from 0 (no rejection) to 3 (severe rejection) in each of three different aspects (parenchymal, biliary, and vascular) will be considered in the "rejection" category. The remaining patients—those whose biopsies were negative for rejection or in whom there was no clinical concern for rejection and therefore no biopsy—will be considered in the "no rejection" category. In general, liver transplant recipients are only biopsied if there is clinical concern, unless they are HCV positive, in which case they are biopsied at routine intervals, and small bowel recipients are additionally biopsied routinely at certain intervals post-transplant.

4. Study Drugs or Devices

There will be no devices or drugs

5. Study Instruments

Flow cytometry will be carried out on an LSR-II flow cytometer.

6. Study Subjects

Subjects will come from one of two cohorts: the CUMC CCTI sample repository or the A2ALL consortium

7. Recruitment

Patients are not being recruited as the study uses already collected samples.

8. Informed consent process

Additional informed consent will not be needed, as this is secondary use of stored samples and health information

9. Confidentiality of Study Data
All blood samples will be received coded. After screening and based on the chimerism results, these samples will be linked to the associated health information as stored by CCTI Sample Repository. Patients will be de-identified during the analysis, with the code being contained in a password-protected secure network.

10. Privacy Protections
All subjects' privacy has already been protected as they previously consented to storage of their blood samples and health information based on IRB#AAF2395 consent form

11. Potential Risks
There is less than minimal risk to subjects as there will be no direct contact with subjects.

12. Data and Safety Monitoring
As above

13. Potential Benefits
This study will possibly yield insight into the mechanisms of allograft tolerance, which may result in strategies that obviate the use of long-term immunosuppression, a major contributor to post-transplant morbidity and mortality.

14. Alternatives
Not applicable

15. Research at External Sites
Not applicable

16. Columbia as Lead Institution
Columbia will be the only research site

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