A Prospective Study of Quantitative CD40 Immunofluorescence and T Cell Receptor Family Subtype in Patients after Orthotopic Heart Transplantation.

Sean Mazer

A. Study Purpose and Rationale

In recent years despite developments in immuno-suppression for heart transplantation, long term survival has not changed over the last two decades. Recent developments in basic immunology have suggested several novel targets for therapy. One of these targets is the CD40-CD40 ligand receptor pathway. CD40 is a cell surface receptor expressed on a multitude of immunologically active cells including B cells, dendritic cells, macrophages and many others. CD40's ligand (CD40L or CD154) is a relatively exclusive cell surface receptor expressed for a brief time on CD4+ T cells, platelets and endothelial cells after activation. For T cells the CD40-CD40L interaction is the requisite second step after the trimeric T cell receptor-MHC II-peptide interaction. The CD40-CD40L interaction rescues the CD4+ T cell from apoptosis and stimulates the antigen presenting cell to differentiate. In T cells interference with this interaction causes T cell anergy to a specific antigen. Animal models of monoclonal anti-CD40L have demonstrated potential as a therapy to treat rejection in heart transplants.[1] Very little is known about how the monoclonal antibody works in transplant models, because our understanding of the effects of solid organ transplant on the immune system is rudimentary.

The molecular technique of immunofluorescence has allowed investigators to probe the expression of CD40 and CD40L in renal allograft rejection and other inflammatory conditions.[2, 3] Quantitative analyses of CD40 expression are an excellent marker of general inflammation. In the transplanted heart Reul et al found that CD40 and CD40L expression on microvascular endothelial cells and infiltrating T cells correlates closely with acute histologic rejection in serial biopsies.[4] The infiltrating T cells which ultimately are responsible for rejection remain poorly studied. The CD40 and CD40L expression have never been studied in chronic rejection.

In heart transplantation the failure of the grafted organs is multi-factorial, yet a syndrome of diffuse Graft Coronary Arteriosclerosis (GCA) has emerged as the most common and difficult to treat cause of transplant failures.[2] GCA may account for up to 40% of all failed hearts (deaths or re-transplantations) occurring within 5 years of transplantation.[3] As many as 20% of patients have evidence of GCA at one year. It is morphologically distinct disease from general atherosclerosis. GCA is a concentric, diffuse intimll hyperplasia, lacking foam cells and atheromata. It affects smaller distal-most arteries including the intra-mural arteries. GCA has a characteristic appearance on cardiac catheterization and is best diagnosed using the technique of intra-vascular ultrasound. The histopathology of the diseased vessels in this syndrome is that of a small vessel vasculitis.[4] Indirect examinations of this process have implicated a cell-mediated immune process as the cause of this syndrome.[5] GCA's histopathologic similarity to other vasculitides and its occurrence in an allograft organ raise the question of whether GCA may be an antigen driven CD4+ T cell vasculitis.

This study's purpose is to learn more about this vasculitis and to attempt to find molecular predictors of its development so that its development may be short-circuited. To this end the study aims to take biopsy specimens from recently transplanted patients and examine them for CD40 and CD40L expression, and T cell receptor monoclonality, and to attempt to correlate this information with the development of GCA. This is the first step in the process of discovering the exact nature of the vasculitis and perhaps a more specific therapy than the broad immuno-suppression currently used.

B. Hypotheses

- The average quantity of CD40 and CD40L expression from several biopsy specimens will correlate with the development of GCA at one year in transplanted hearts.
- The T cell receptor phenotypes from patients who develop GCA will be distinct from those who do not reflecting an antigen driven vasculitis.

C. Study Design

This study is a prospective cohort study designed to examine the correlation of molecular markers with the development of a primary outcome. The study will be done only at NYPH. Qualifying patients who consent will be enrolled after surgery, prior to discharge home. Three biopsy specimens will be collected from the patients at months 3,4, and 5 as part of their routine biopsy protocol post-transplantation. These times were chosen to minimize crossover between acute cellular rejection and chronic rejection. The biopsies will be done as part of the routine transplant evaluations and additional specimens are not required. The molecular analysis of the tissue will be done after the routine pathology readings. The specimens will be stained for CD40 and CD40L. Two independent pathologists will score the biopsies from 0-3 based on the CD40 staining pattern. CD40 is the primary measure of interest.

As secondary variables of interest, the investigators will also collect information about co-morbidities that could contribute to the development of GCA starting from the time of transplantation:

T cells from the biopsy specimens will be immortalized and their T cell receptor phenotype will be analyzed.

Presence of type II diabetes, severity and control after transplantation, as measured by pre and post- transplant glycosylated hemoglobin

Lipid profile

CMV status

Number of rejections in the first 3 months requiring treatment (biopsy score of 1 with hemodynamic compromise or 2 without hemodynamic).

All patients will be followed through their one-year post-transplantation examination. At this point they normally undergo cardiac catheterization for atherosclerosis evaluation. To increase the accuracy of assessing for GCA, an intra-vascular ultrasound of the coronaries will be done. The intra-vascular ultrasound is positive if the patient has an increase in intimal thickening of >0.5mm from baseline on one or more of the horizontal cuts.

D. Statistical Analysis

For the CD40 and CD40L expression data, the investigators will collect data in biopsy specimens from months 3,4, and 5. The biopsies will be stained with anti-CD40 and anti-CD40L antibodies. Two separate pathologists will score the biopsies from 0-3 using a scoring system which other investigators have used quantitatively.[2] For both molecules the three biopsy scores will be averaged to produce a single score. This should lessen the sampling error inherent in right heart biopsies. If the CD40 data is normally distributed, then it will be analyzed using the T test. If not then a Wilcox regression model will be used. For CD40L the biopsies will be determined to either have expression or not. As CD40L is a qualitative variable Chi square analysis will be used to evaluate its correlation with GCA.

To analyze the T cell data, the each subject's T cell receptor Vß subtypes will be plotted. If the pattern is oligoclonal or monoclonal suggesting a limited antigen driven process, then they will be determined to be positive. If the pattern is polyclonal, then they will read as negative. The T cell receptor data will be analyzed using a Chi square model to assess correlation with the presence of GCA. At 12 months all patients will undergo intra-vascular ultrasound. Two qualified readers independently will read these and will interpret the results using the definition of a positive IVUS. The other secondary endpoints' relationship to GCA will be compared using T tests.

The study is powered using the CD40 as the independent variable. To make this calculation we must use other models to try to make a reasonable prediction of the studies' results. Fortunately, chronic rejection in kidney transplant is an excellent model GCA and this power calculation in particular for several reasons.:

Transplanted kidneys share a similar histopathologic pattern of chronic rejection with heart transplants involving the renal vasculature.

CD40 and CD40L staining has been done in chronically rejecting kidneys.

The power of this study to detect an association between CD40 staining and GCA is set at 80%. Given the following assumptions, 80% of the patients be effected at one year (based on clinical data), with sigma=0.31 and delta= 1.0, 25 patients will be required.[10] Approximately, 90 transplants/year are done at this institution. With this in mind, the entire study, the from the first patient's enrollment to the completion of the last patient's one year follow-up, will take about 15 months.

E. Study Procedures

This study will enroll patients during the post-operative period before discharging the patient home. Data about the patient/graft's MHC compatibility and their post-transplantation regimen will be collected. At the 3, 4 and 5 month transplantation right heart catheterizations, the pathologists will read the patients' biopsies for rejection. The biopsies will then be stained for stained for CD40 and CD40L and quantitatively analyzed. Two pathologists will independently score the biopsies from 0-3. Then CD4+ T cells will be cultured when possible from the biopsy specimens and immortalized. RtPCR and other molecular techniques will be used to establish the Vß subtype of each T cell receptor. At one year the patients will also undergo intra-vascular ultrasound (IVUS) of their coronary arteries along with the normal cardiac catheterization to assess the development of GCA. IVUS is the most accurate method of assessing the extent of GCA present in the transplanted heart. Data about global function will be collected during the catheterization including ejection fraction and cardiac index.

F. Study Drug

N/A

G. Medical Devices

N/A

H. Study Questionaire

N/A

I. Study Subjects

The study subjects will be patients who have undergone transplantation at New York Presbyterian Hospital. They will be enrolled consecutively after the heart transplant when they are ready for discharge.

Inclusion criteria for the study will be the same as for the transplant.

- Patients without a known rheumatologic disease
- Age 16-69 years who have had a heart transplant
- Patients who have been consented to the study

Exclusion criteria:

- Patients who have obvious donor coronary disease documented by angiography at the time of transplantation, or who have undergone revascularization for donor atherosclerosis.
- Patients who suffer acute rejection prior to discharge home
- Patients who are enrolled in another trial to treat GCA
- Patients with white blood cell counts >4,000/cc at discharge
- Patients transplanted with ABO incompatible hearts
- Patients whose creatnine is >2.0 at the time of discharge

J. Confidentiality of Study Data

The patients' clinical and pathologic characteristics will be kept strictly confidential. Their information will not be stored with their names and

K. Location

The enrollment of patients will take place in the Heart Institute of the Milstien Hospital. The biopsies will be done per the protocol in the transplant biopsy suite, and the intra-vascular ultrasound will be done in the Cath Lab.

L. Conflict of Interest

N/A

M. Risk to the Patient

The only risk to the patient is the performance of the intra-vascular ultrasound. The clinicians here have a great deal of experience with this procedure having been involved in several large trials which require it. This substantially reduces the risk. The patients will not have to undergo an additional percutaneous stick for this since it can be done through the standard introducer.

N. Benefit to Patient

New therapies like Rapamycin and others are being developed for the treatment of GCA. The patients who enroll in this will ultimately receive a more thorough evaluation for the presence of GCA than is normally undertaken. This may allow early treatment of some of their GCA and ultimately prevent morbidity. The additional evaluation that they will receive is:

More intensive molecular examination of review of their three biopsy specimens.

An IVUS evaluation at one year. The IVUS is a superior means of evaluating the extent of GCA.

Information obtained during the study will be made available to the patients' physicians and the patients.

O. Alternative Therapy

N/A

P. Compensation to Subjects

N/A

Q. Cost to Subjects

N/A

R. Minors as Research Subjects

N/A

S. Radiation or Radioactive Substances

N/A

T. Importance

The incidence and consequences of developing GCA make the stakes very high in this disease. Currently much is known about the natural history of GCA, but its cause remains obscure. This study could provide evidence of a T cell mediated antigen driven vasculitis causing GCA. This discovery could lead to the development of novel therapies for GCA, perhaps even a cure. The techniques to probe the T cell physiology in transplantation are new and using them in this study should increase our understanding of the immunologic state in the transplanted heart and provide valuable experience applicable to other solid organ transplants.

U. References:

1 Chang AC, Pierson RN 3rd et al, "Prolonged anti-CD40 ligand therapy improves primate cardiac allograft survival," Transplantation Proceedings, 31(1-2):95, Feb-Mar, 1999.

2 Gaweco, AS, Van Thiel, D et al, "CD40 expresssion of graft infiltrates and parenchymal CD154 (CD40L induction in human chronic renal allograft rejection," Kidney International, 55:1543-1552, 1999.

3 Yellin, M, Chess, L et al, "Immunohistologic analysis of Renal CD40 and CD40L expression in Lupus Nephritis and other Glomerulonephritides," Arthritis and Rheumatism, 40(1):124-34, January 1997.

4 Reul RM, Ganz P. Briscoe DM et al, "CD40 and CD40 ligand (CD154) are coexpressed on microvessels in vivo in human cardiac allograft rejection," Transplantation. 64(12):1765-74, Dec 27, 1997.

5 Young JB, "Allograft vasculopathy: diagnosing the nemesis of heart transplantation [editorial; comment]," Circulation, 100(5):458-60, August 1999.

6 Gao S, and Stinson EB. Prevalence of accelerated coronary artery disease in heart transplant survivors: comparison of cyclosporine and azathioprine regimens," Circulation, 80(suppl III):III-100-III-5, 1989.

7 Higuchi, M., Libby, P. et al, "Histological Evidence of Concomitant Intramyocardial and Epicardial Vasculitis in Necropsied Heart Allografts," Transplantation, 67(12):1569-76, June 1999.

8 Hosenpud JD, Wagner CR, et al, "Cardiac allograft vasculopathy:association with cell-mediated but not humoral alloimmunity to donor-specific vascular endothelium," Circulation. 92:205-211, July 1995.