New possibilities to improve the outcomes of renal transplantation

Ph.D. Thesis

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I. ACUTE ANTIBODY MEDIATED REJECTION (AMR)

1. INTRODUCTION

The number of patients awaiting transplantation has been increasing due to advances in immunosuppression while there is no good solution to expand the number of donors and to increase the number of transplantations. One possibility to ease the need for renal transplantation is to increase the life span of grafts. Better maintenance therapy as well as earlier diagnosis and effective treatment of rejection can save allografts by minimizing damage.

Substantial proportion of acute and chronic renal allograft rejection processes is caused by antibodies reactive to donor antigens. Antibody mediated acute rejection (AMR), a newly described entity, arises despite ongoing therapy with potent anti-T cell pharmacological agents. AMR, the term is used to describe all rejection reactions involving donor-specific antibody (DSA) or donor reactive antibodies, e.g. toward non-donor foreign anti-human leukocyte antigens (HLA), ABO isoagglutinins, and anti-endothelial antibodies. AMR results from the interaction of anti-donor antibodies, which are either preexistent at low titer or developed de novo after transplant, with allograft vascular endothelium. It can be mediated directly by *complement.* The finding of complement fragment C4d in rejecting graft peritubular capillaries provided strong evidence to support this theory. *Antibody-dependent cell toxicity* may also play a role in mediating endothelium damage without the involvement of complement.

Traditional anti-rejection treatments, such as administration of steroid boluses or anti-lymphocyte antibodies are usually ineffective to treat AMR, since these modalities are primarily directed toward cellular immune mechanisms. Approaches to treat AMR seek to remove deleterious antibodies and other factors such as complement and cytokines using plasmapheresis (PP) or immunoadsorption, and to modify B cell activation and antibody generation by

administration of intravenous immunoglobulin (IVIg) or low-dose CMV hyperimmune globulin and to eliminate B-cells with rituximab.

CD20 is a B-cell surface antigenic phosphoprotein that is restricted in its expression to pre-B and matures B-cells. It is neither present on stem cells nor on plasma cells. The following major effects of rituximab contribute to its *B cell depletion properties:* antibody dependent cell mediated cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), apoptosis induction in CD 20+ B-cells and direct growth arrest.

A retrospective analysis of 54 kidney transplanted patients was conducted at the University of Texas, Health Science Center at Houston to compare the outcomes of a plasmapheresis (PP)-based versus a PP plus rituximab regimen to treat patients experiencing AMR and resistant to steroid plus anti-lymphocyte globulin treatments.

2. MATERIALS AND METHODS

Patients were grafted between 2001 and 2006, a period during which 568 grafts were performed. This experience included 26 patients (Group A) who received PP plus rituximab which was initiated after the inferior experiment results among an initial 28 patient (Group B) cohort who underwent PP without rituximab. IVIg supplementation was administered if the IgG or IgM levels were below the level of normal limits.

All renal transplants were performed following a negative cross-match by complement dependent cytotoxicity (CDC) enhanced with antihuman globulin (CDC-AHG) and flow cytometry using a fluorescein conjugated anti-human immunoglobulin reagent. Anti-Class I and Class II HLA PRA determinations were performed before transplantation on all patients using

CDC-AHG and flow cytometry techniques. DSA was also evaluated by microbead technology in flow cytometry with goat anti-human IgG-phycoerythrin.

Diagnosis of AMR was based on the clinical course, histological characteristics, C4d staining and presence of DSA-s in recipients serum.

The typical treatment protocol was a two week course: five daily plasma exchanges followed by a two day rest. Thereafter, thrice weekly procedures were performed for two to three weeks depending upon whether there had been a reduction of at least 30% in the serum creatinine. At the end of each PP cycle, the patients in Group A were administered rituximab (Rituxan, Genentech Inc., South San Francisco, CA, USA; 375 mg/m^2).

3. RESULTS

There was no significant difference in the baseline demographic characteristics (age, gender, source of transplant, panel reactive antibody, HLA mismatch, diabetes, prior blood transfusion, ethnicity, etiology) between the two groups. All patients had undergone induction therapy, but there was no difference in the proportions of Group A versus Group B subjects treated with anti-thymocyte polyclonal globulin or basiliximab induction therapy. The baseline immunosuppressive regimens included significantly more cases treated with CsA de novo among Group B (p=0.04). The median values of the time to rejection as well as the occurrences of AMR with or without ACR did not show significant differences. Among cohorts A and B, 14 and 15 patients had experienced an ACR episode prior to the diagnosis of AMR, respectively. All patients received initial therapy with monoclonal or polyclonal anti-lymphocyte immunoglobulin antibodies at similar mean doses prior to recognition and during treatment of the AMR. Significantly more Group A patients received IVIg (IVIg/Cytogam; Fisher's exact test, *P*=0.02)

as well as a greater number (P=0.003) and length (P=0.009) of PP treatments to treat AMR. Despite these differences, the multivariate analysis documented that only the prescription of rituximab was a major factor to improve outcomes. To evaluate independent risk factors for 2-year renal allograft survival, a multivariate analysis was performed using a Cox regression model. The relative risk of graft failure at 2 years was 5-fold greater for patients who were not treated with rituximab; none of the other variables showed a significant impact.

Patient survival rates among Group A versus Group B were 100 versus 90% (P=NS), respectively. The respective overall graft survival rates at 2 years were 90 versus 60% for Groups A versus B (P=0.005), respectively. Upon multivariate analysis the administration of IVIg or Cytogam alone was associated with improved graft survival compared with non-treated, PP only subjects (90% vs. 66%, P=0.05). However, administration of rituximab yielded significantly better graft survivals (92% vs. 60%, P=0.009). Even greater outcomes were obtained with the combination of rituximab and IVIg/Cytogam compared with non-treated, PP only subjects (94% vs. 53%, P=0.025).

MDRD estimates of GFR values reflecting the allograft function showed no significant difference over time (General Linear Model, Repeated Measures test, P=0.42) between the groups.

Among the 54 patients who underwent 838 plasma exchange procedures, the 124 complications occurred in 29 patients, who were similarly distributed between groups A and B. Infectious complication(s) within 6 months showed no significant impact on graft survival at 2 years, using the Kaplan-Meier method (P=0.66, Log-Rank test). Furthermore, there was no significant difference regarding the rates/types of infectious complications between the two

groups within 3 or 6 months after completion of treatment (P=0.24 and P=0.78, respectively). Administration of IVIg showed a trend toward decreasing the appearance of infectious complications in Group B (P=0.058) but not in Group A (P=0.42).

4. NOVELTIES

4.1. This retrospective study showed a 2-year graft survival of 92% for patients treated with rituximab plus plasmapheresis, which was significantly greater (Log-Rank test; p=0.025) than that observed among the group who did not receive anti-CD20 therapy.

4.2. The 100% patient survival at 2 years as well as the absence of a greater incidence of major complications among individuals treated with rituximab supports the effective, safe use of this monoclonal antibody for AMR.

4.3. There was no significant difference regarding the rates/types of infectious complications between the two groups within 3 or 6 months after completion of treatment (P=0.24 and P=0.78, respectively). Administration of IVIg showed a trend toward decreasing the appearance of infectious complications in Group B (P=0.058) suggesting the beneficial effect of it.

4.4. C4d staining and DSA detection and monitoring seem to be very helpful markers for the diagnosis of acute antibody mediated rejection.

5. CONCLUSION

Our work at The University of Texas, Division of Immunology and Organ Transplantation has revealed the primacy of rituximab in the treatment of AMR besides the previously established therapeutic effect of plasmapheresis and IVIg. The 100% patient survival

at 2 years as well as the absence of a greater incidence of major complications among individuals treated with rituximab supports the effective, safe use of this monoclonal antibody for AMR.

II. POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD)

1. INTRODUCTION

PTLD has been broadly defined as a benign or malignant lymphoid proliferation that develops as a consequence of pharmacological immunosuppression following the solid organ or bone marrow transplantation. Its occurrence among engrafted patients is 12- to 20-fold higher than that among the general population. The majority of PTLDs are EBV-associated, of B-cell origin, and expressing CD20 antigen. The pathogenesis of EBV-associated PTLD is linked to T-cell dysfunction. The greatest risk of developing PTLD occurs during the first year after transplantation. Among solid organ transplant recipients, the median time of onset of PTLD is about 6 months. There seems to be a relationship between the occurrence of PTLD and treatment of rejection episodes with increased immunosuppression. The typical evolution of disease occurs in patient on calcineurin inhibitor therapy who suffers a rejection episode which either does not respond to steroid or rapidly relapses after tapering steroids, requiring treatment with one or two courses of antilymphocyte globulin or OKT3. Shortly thereafter the patient develops PTLD.

Clinically, PTLD can present in a number of ways: 1. oropharyngeal hyperplasia or lymphadenopathy which resembles infectious mononucleosis; 2. a fulminant, rapidly progressive polyclonal lymphoid hyperplasia; 3. or most commonly as a single or metastatic polyclonal or clonal tumors which most often is observed in an extranodal locations within the brain, gastrointestinal tract, or allograft. Approximately 90% of patients show an association of active EBV infection, either primary or by reactivation.

When detected in an early state, reduction in immunosuppression is an effective therapy, with high response rates. Targeting EBV by antiviral agents such as ganciclovir or acyclovir has been attempted for prophylaxis and treatment of PTLD. Non-specific immune stimulants such as interferon-alpha can enhance immune system in PTLD patients. Chemotherapy is the standard salvage therapy after failure of reduction in immunosuppression, but carries significant mortality and morbidity rates in the organ transplant population. Rituximab was first approved for the treatment of relapsed low-grade CD20-positive non-Hodgkin lymphomas with reported overall response rates of up to 50% and complete remission rates of 5%. Rituximab also has an expanding role in management of various non-malignant diseases, especially autoimmune condition in which B-cells play important role, including rheumatoid arthritis, Sjogren's syndrome, systemic lupus erythematosus, myasthenia gravis, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura and *dermatomyositis, polymyositis*.

Over the recent years many case reports and series described the use of rituximab in PTLD. Phase II trials prospectively confirmed its clinical benefit in PTLD. Most patients also underwent concurrent reduction of immunosuppression, some concurrent antiviral therapy. Many subjects experienced clinical improvements within a few days of the first infusion, but in some instances the benefit was not observed for a few months. Most patients in the case reports were treated with the standard dose of rituximab (375 mg/m2) once a week for four consecutive weeks.

2. CASE REPORT

In the thesis a succesfull treatment of a 65 years old PTLD patient has been reported whose disease was associated with an en bloc renal transplant performed in a patient with a history of recurrent bouts of dermatomyositis. What made it unique is the multiple disturbed

immune system which eventually has led to development of PTLD. Additional risk factors for malignancy were the intense mycophenolate mofetil regimen to control the autoimmune disease, the advanced age of the recipient, CMV mismatch, and an EBV-positive donor. Rituximab and SRL were chosen due to the antibody's favorable actions on non-Hodgkin lymphomas and dermatomyositis. This case also provided evidence for the efficacy of rituximab combined with SRL to treat PTLD — over 90% of neoplastic cells were necrotic —while suppressing activation of the immunologic comorbidities associated with dermatomyositis.

3. CONCLUSION

It can be concluded that the majority of EBV-associated, CD20-positive PTLD patients benefit from rituximab as the second line of treatment right after or besides the reduction of immunosuppression. Our successfully treated patient also provided evidence for the favorable effect of this treatment modality. Given the significant toxicity, chemotherapy is best reserved for use in patients who are ineligible or fail rituximab.

IV. THE ROLE AND EFFICACY OF SIROLIMUS IN RE-TRANSPLANTATION

1. INTRODUCTION

While outcomes following transplantation have improved over the years, allograft loss is a problem ultimately confronted by many recipients. Re-transplantation offers a better survival benefit compared with continuous dialysis after kidney transplant failure. Patients who lose their first grafts have three options: hemodialysis, which results in a poor quality of life and is the least cost effective; peritoneal dialysis which is often complicated by recurrent peritonitis and other intra-abdominal complications but is cost-effective and allows a more active lifestyle; or repeat renal transplantation, which has obvious quality of life benefits, but inherent risks relative to graft survival and is expensive at least in the first year.

While re-transplantation offers hope, previous studies have demonstrated that outcomes following repeated transplantation are, in general, inferior to those observed with first transplants. We have also investigated the influence of demographic and immunological factors on the patient and graft survivals as well as occurrence of acute and chronic rejections among primary and re-transplant patients.

2. MATERIALS AND METHODS

Between May 1994 and November 2005, a cohort of 162 (15%) subjects underwent renal re-transplantation within the overall population of 1,062 grafts at the University of Texas, Division of Immunology and Organ Transplantation. Within this cohort 98 (64%) received *de novo* sirolimus-based immunosuppression. None of the re-transplant patients had been previously treated with sirolimus. The 900 patients who underwent primary transplantations included 576 (64%) who were enrolled in *de novo* protocols of sirolimus immunosuppression. From these 576 subjects we selected a control population of 200 patients who were matched to the 98 repeat graft recipients based upon month of grafting and demographic features

3. RESULTS AND NEW FINDINGS

3.1. Upon univariate analysis the risk factors for graft loss included prior transplant loss within 6 months (P=0.0001), older mean recipient age (P=0.01), occurrence of an BPAR (P=0.049) and donor ethnicity (P=0.05). The use of living donors did not appear to yield better results. Upon multivariate analysis, graft loss at 5 years was significantly increased among recipients who experienced BPAR (P=0.034, HR 2.42). Patient survival at 2, 3 and 4 years showed the benefit of recipient age <60 years (P=0.033, HR 0.185; P=0.05, HR 0.22; P=0.05, HR 0.22), and at 5 years, the absence of diabetes mellitus (P=0.034, HR 0.037). Freedom from an acute rejection episode at 5 years tended to be associated with an HLA mismatch <3 (P=0.07, HR 0.164).

3.2. In our experience there was no greater incidence of surgical complications among second procedures. Furthermore, we neither confirmed attenuated survivals of repeat kidney grafts among women undergoing second versus first transplantations, nor of re-exposure to foreign HLA antigens present on the prior graft.

3.3. Patient survival rates for primary versus re-transplant cases at 1 year (96 versus 94%; *P*=0.49) and at 5 years (88 versus 86%; *P*=0.68) were not significantly different. The graft survival rates at 1 year (90 versus 90%; *P*=0.96) and 5 years (78 versus 77%; *P*= 0.92) were also comparable (Log-Rank, *P*=0.98).

3.4. Incidences of biopsy proven acute rejection and chronic rejection/chronic allograft nephropathy were similar between the cohorts (Log-Rank, *P*=0.12 and *P*=0.99, respectively). Among the repeat transplants there were 5 (5%) humoral-vascular rejection episodes compared with 8 (4%) among the primary grafts.

3.5. The post-transplant serum creatinine levels at 1, 3, 12, 24, 48 and 60 months among repeat versus primary recipients were not significantly different. Kidney function at these times was also similar for both populations, as computed by the abbreviated MDRD formula.

5. CONCLUSION

The novelty of our findings was that a sirolimus-based regimen yielded similar efficacy and outcomes among re-transplanted patients compared with first renal transplantations with a mean 5-year follow-up.

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American Transplant Congress

Accepted poster

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