FOXP3+ T-regulatory Cells in Renal Transplantation as a Novel Tolerogenic Marker Predicting Outcomes: Histologic, Immunohistochemical and Urine PCR Correlates

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1. Introduction

Renal Transplantation is performed for eligible patients with end-stage renal disease (Suthanthiran 2000) showing irreversible kidney failure. Without renal replacement therapy (dialysis or kidney transplantation), complications are multiple and severe, and death may follow (Cattell et all. 1998). Since Blood Group and Human Leukocyte Antigens (HLAs) are the primary cause of stimulation for the initiation of the immune response after transplantation (Suthanthiran 1993), they must be closely matched in order to prevent an immune mediated rejection of the donor renal allograft in the recipient (Suthanthiran et al. 1994). Acute rejection is the most frequent post-transplant clinical complication (Li et al 2001), defined as deterioration of renal allograft function. It is considered to be a significant risk factor for chronic rejection, the most common cause of long-term allograft failure (Sharma et al. 1996).

The immunobiology of renal allograft rejection involves the coordination of antigenic stimulation and costimulatory signaling (Suthanthiran 1997). Antigenic stimulation occurs when recipient T-cells are activated (cytotoxic T cells or CTLs) during recognition of antigen of the major histocompatibility complex proteins (MHC) on the donor cells, that are expressed on the surface of the antigen presenting cells (APCs) (Suthanthiran 1996). The T cell antigen receptor (TCR/CD3) cell surface molecule complex) is a costimulatory signal involved in T cell activation and their proliferation (Suthanthiran 2000). This leads to transcription of IL-2 and other significant T-cell activation genes (Suthanthiran 2000) to elaborate cytokines such as granzyme B, perforin, Fas Ligand, and tumor necrosis factor (TNF-a), which are directly involved in injuring target cells in the allograft, together with immunoregulatory cytokines such IL-2, IL-4, IL-10, IFN-gamma and transforming growth factor (TGF-b) (Suthanthiran et al. 2001).

Standard diagnosis of acute rejection is based on the invasive procedure of needle core biopsies of the allograft kidney, which has limitations. Recently, a noninvasive procedure that may aid in early diagnosis of rejection was reported. This is a quantitative polymerase chain reaction (PCR) assay (Li et al. 2001) to detect mRNA of perforin and granzyme from cytotoxic T cells in urine samples, increasing the diagnostic accuracy of allograft rejection.

Extensive basic and clinical research has identified that, in this cascade of immune reactivity, there are possible target sites within the various immune pathways that can be inhibited by immunosuppressive drugs, to treat an acute allograft rejection (Suthanthiran et al 1996 and 2000). They include prednisone, azathioprine, cyclosporine, tacrolimus, sirolimus (rapamycin), and mycophenolate mofetil (MMF). Combinations of different drugs, e.g. Cyclosporine/Tacrolimus, MMF and prednisone are effective drug regimens to intervene in more than one site in the immune pathway leading to rejection. Other therapeutic options include monoclonal antibody (mAb) anti-cytokine therapies (Skurkovich et al. 2002). In addition, further research is directed to find more effective means of achieving stable graft function (Suthanthiran 2000).

Previous studies have also shown intrarenal expression of cytotoxic T cells and cytokines in tissues, correlating with acute rejection (Suthanthiran 1997). Reverse transcriptase-polymerase chain reaction (RT-PCR) permits the quantitative detection of intrarenal messenger RNA (mRNA) of cytotoxic attack molecules (granzyme B and perforin), and immunoregulatory cytokines (TGF-*B*, IFN-*y*, IL-10, IL-2 and IL-4) in urine in small quantities. Other proteins associated with acute rejection, such as interferon-inducible-protein-10 (IP-10), CD103 (alloreactive CD8 &T cells) and chemokine receptor CXCR3, have also been identified in urinary cells (Ding et al. 2003).

Dendritic cells (DCs) are potent antigen-presenting cells (APCs), among others, and are also known as the sentinels of the immune response which induce and regulate T-cell reactivity (Lagaraine et al. 2003). There are two types of DCs: myeloid and plasmacytoid. Myeloid DCs are more potent in the immune response, whereas the plasmacytoid DCs are more active in achieving tolerance. They are controversial as APCs, because they exhibit multiple properties in different states such as tolerance vs. rejection (Ardavin et al. 2004). Immature DCs must receive a “danger” signal to become a mature immunogenic DC or a “tolerizing signal” to become a tolerogenic DC (Santiago-Schwartz 2004). Following transplantation, DCs present antigens to T cells via the direct or indirect pathways of the immune system and may be the primary instigators of transplant rejection. (Morelli et al. 2003). They can express both class I and class II MHC molecules required for the presentation of antigen to T cells along with appropriate costimulation such as CD80 (B7-1) and CD86 (B7-2), required for T cell stimulation (Fierro et al. 2003).

The ultimate goal of organ transplantation is to establish graft tolerance that is as close to natural self-tolerance as possible. Transplant tolerance is defined as an ability to accept a renal allograft in the recipient, counter the donor organ attacks and initiate a destructive immune response. Tolerance is antigen specific and may be induced because of prior exposure to the specific antigen (Suthanthiran 1996). The deletion of the T and B lymphocytes, suppressor and anergy mechanisms have been advanced as the basis for tolerance to self-antigens and the avoidance of autoimmunity (Womer et al. 2004). In the normal state, the process of clonal deletion occurs when self-antigen reactive cells are eliminated from the immune system. Antigen-specific T or B cells are present and functionally capable in tolerant states, but are restrained by the suppressor cells (Suthanthiran 1995).

Regulatory T cells (T-reg cells), which play a significant role in tolerance induction after transplantation, have also been shown to inhibit autoimmunity and inflammatory diseases (Chai et al. 2005). T-regs release a signal that directly impedes the APC from stimulating other T cells, thus, suppressing the activity of the APC and releasing molecules that inhibit T cell activation and proliferation, T-regs naturally arise from CD4/helper T cells, expressing CD25. Prior studies have indicated a critical role for CD4+CD25+T-regs in immunologic tolerance. T-reg cells can be generated from thymus or can differentiate from CD4+CD25- naïve T cells. The identification of a T-reg marker resulted in the discovery of *FOXP3*, which is the transcription factor that encodes a repressor gene, Scurfin (Chai et al. 2005). *FOXP3* belongs to the X-linked, forkhead-winged helix family and is the master regulatory gene controlling and initiating the development and function of T-reg cells, specifically from naturally arising CD4+CD25+ cells which are capable of inducing self-tolerance (Chai et al. 2005). Recent experiments showed that TGF-*B* and TCR costimulation converts naïve CD4+CD25- T cells into suppressor T cells by inducing *FOXP3* expression (Chen et al. 2003).

A recent study reported the expression of mRNA for *FOXP3* in urinary cells of renal allograft recipients in predicting outcomes of acute rejection. The original hypothesis predicted that there would be a higher level of *FOXP3* in stable functioning grafts and lower levels of *FOXP3* in acutely rejecting allografts. The levels of *FOXP3* mRNA in urine revealed significantly higher levels in allografts with better outcome, and lower levels in those with irreversible acute rejection and graft failure (Muthukumar et al. 2005).

The present study aims to determine the magnitude and relationship of tissue expression of T-reg cells and DCs in stable functioning grafts and acutely rejecting renal allografts evoking two main hypotheses.

Hypothesis 1: In stable renal allografts, the number of tissue CD4+, CD25+, *FOXP3*+Treg cells will be increased than those with acute rejection.

Hypothesis 2: T-reg cell activity in the renal allograft is dependent upon DC signals and functions following transplantation in the kidney.

2. Materials and Methodology

This research involved different laboratory techniques, including Quantitative Real-Time PCR and Immunohistochemistry, completed by laboratory technicians/scientists. This research had the following objectives: Detection of infiltrating cell types (FOXP3, DC) using specific antibodies in diagnostic renal biopsy tissue, identification of various gene expressions in the urinary cells and integration of histologic, immunohistochemical, PCR and clinical data.

This researcher examined a total of 50 kidney transplant biopsies from 50 patients for this study. These biopsy specimens were fixed in 4% paraformaldehyde, treated routinely in a tissue processor and embedded in paraffin wax. The sections were then stained with hematoxylin and eosin, periodic acid-Schiff (PAS), and Masson's trichrome. Each renal biopsy was evaluated by Light Microscopy (LM), using an Olympus binocular microscope, systematically examining glomeruli, the tubulointerstitial compartment and blood vessels, which was previously reported by a renal pathologist. During re-evaluation of LM slides by this researcher, the number of glomeruli per case (mean $\pm$ SD; range) was recorded to determine the adequacy of the biopsy specimen.

2.1. Polymerase Chain Reaction

Kinetic Quantitative PCR Assay

Urine PCR data, for CD25 and *FOXP3* mRNA levels in these 28 acute rejection allografts and 22 stable graft patients, was obtained from the previous study reported by this laboratory (Muthukumar et al 2005). The mRNA was measured with kinetic quantitative polymerase chain reaction (PCR) assay. The urine samples were centrifuged at 10,000g for 30 min. at 4 degrees Celsius. RNA was isolated from urinary cell pellets using a RNeasy minikit, quantified and reverse transcribed to complimentary DNA (cDNA). Laboratory scientists (Drs. Li and Ding) designed and synthesized fluorogenic probes and oligonucleotide primers for the measurement of mRNA levels of *FOXP3* and *CD25.* The kinetic quantitative PCR assay analysis was performed by a two-step process, a pre-amplification step followed by the measurement of mRNA with an ABI Prism 7700 system. The pre-amplification reaction was set up in a PTC-200 thermal cycle with 3 microliters and 7 microliters of dNTP, Taq DNA polymerase, 10X PCR buffer, and gene specific oligonucleotide primer pairs. For each urine sample, the PCR assay was arranged in copies in a 25 microliter reaction volume using 12.5 microliters TaqMan Universal PCR Master Mix, 2.5 microliters in pre-amplified template cDNA, 300 nM primers and 200 nM probe. This protocol included 40 cycles of denaturing at 95 degrees Celsius for 15 sec and primer annealing and extension at 60 degrees Celsius for 1 min (Muthukumar et al 2005). Transcript levels were calculated by standard curve method, which is based a principle that a plot of the log of the original target copy number of a standard versus threshold cycle results in a straight line. The mRNA levels in the urine samples were expressed as number of copies per microgram of total RNA isolated from the urinary cells (Li et al 2001). This researcher spent time in Dr. Suthanthiran's laboratory to observe and learn the above technique on urine samples during the last three years.

2.2. Immunohistochemistry (IHC)

Specimen Preparation

Immunohistochemical procedures were performed on paraffin embedded renal biopsy tissue. The tissue sections were deparaffinized and hydrated using a standard procedure. To eliminate endogenous peroxidase activity and background staining in the tissue, the sections were incubated in 3% hydrogen peroxide solution in methanol for ten minutes and washed in phosphate buffer.

Immunohistochemical Staining

This portion of the antibody staining was performed by an autostainer (Bond-max), which uses a polymer defined detection system (Vision Biosystems). This requires antigen retrieval to expose the antigenic epitope by pre-treating the tissue sections with a solution known as BD Retrievagen. At appropriate pH for each antibody used and length of time (noted below), they are incubated under intense heat for 10 min. and slowly cooled to room temperature for 20 min. After each primary antibody was diluted in the antibody diluent for IHC, the sections were covered by mouse monoclonal antibody *FOXP3* (High pH 8.0 for 20 min; 1:100 dilution; Abcam), mouse monoclonal antibody CD25 (High pH 8.0 for 20 min; 1: 300 dilution; Weill Cornell Labs, New York, NY), mouse monoclonal antibody CD83 (1:20-1:40 dilution; Abcam) and mouse monoclonal antibody DC-LAMP (enzymatic pretreatment of proteinase K for 15 min; 1:40 dilution; Beckman Coulter) and incubated for 1 hour.

To ensure favorable antibody penetration and adequate staining, the biotinylated secondary antibody was diluted in the antibody diluent for IHC, applied to the tissue sections and incubated for 30 minutes at room temperature. After the application of the pre-diluted streptavidin horseradish peroxidase, the tissue sections were incubated for 30 min at room temperature. All the slides were rinsed in three 2 min PBS buffer washes, after every step. Next,

the sections were developed for the positive reaction using diaminobenzidine (DAB). The DAB substrate solution was prepared by adding 1 drop of DAB chromagen to every 1 ml of the DAB buffer. The sections were incubated in DAB until optimal staining was reached, and subsequently washed. The tissue is dehydrated in a routine manner for examination. This researcher personally spent time in Dr. Daniel Knowles's immunopathology laboratory observing and learning this technique and handled the slides until the final step with supervision from the laboratory technicians. The number of positive cells in ten (10) consecutive high power fields (HPFs) per antibody (*FOXP3*, CD25, DC-LAMP, CD83) per case was evaluated. The number, distribution and types of interstitial cell aggregates (peri-glomerular, peri-tubular, peri-vascular or peri-venular) were also noted.

3. Results

Twenty-eight (28) renal biopsy specimens, (age range, 20-64; age, 39 $\pm$ 11 [mean $\pm$ SD] ; 17 women and 11 men; 9 living related donor grafts, 8 living unrelated donor grafts, 7deceased donor grafts, and 14 simultaneous pancreatic and kidney grafts) were classified as acute rejection and 22 biopsies (age range 26-65; age, 43$\pm$ 13; 11 women and 10 men; 15 living related donor grafts, 4 living unrelated donor grafts and 1 deceased donor graft) were classified as stable functioning allografts showing no rejection using the 1997 Banff schema for transplant rejection (Racusen et al 1997).

Of the twenty-eight (28) patients with acute rejection, the initial serum creatinine levels at the time of biopsy range from 1.3 to 10.9 mg/dl and the mean $\pm$ SD was (3.55 mg/dl $\pm$ 2.58 mg/dl). Following immunosuppressive treatment, after six (6) months, eight (8) of the twenty-

eight (28) renal allografts progressed to graft loss. Of the remaining patients, the creatinine levels ranged from .9 to 3.8 mg/dl and the mean $\pm$ SD was (1.86 mg/dl $\pm$ .77 mg/dl).

Using the Banff 97 classification, we categorized twenty-eight (28) biopsy specimens that were diagnosed with acute rejection as follows: Eleven (11) showed grade IA (foci of moderate tubulitis), fifteen (15) were grade IB (foci of severe tubulitis), one (1) was grade IIA (mild-moderate intimal arteritis), one (1) as IIB-III (severe initimal arteritis-severe intimal arteritis), and one (1) as II-III (vascular rejection with fibrinoid necrosis). The various parameters of acute cellular rejection: tubulitis (,inflammatory cells infiltration of the tubular lining cells), and foci of tubular atrophy (shrunken and non functioning tubules) as a result of inflammation or other insult, graded interstitial inflammation in the spaces and appearance of small renal arteries and arterioles for inflammation (vasculitis) were observed. While CD25 staining was observed mainly localized in the cytoplasm of the cells, *FOXP3* was observed in the nuclei having a homogenous or granular appearance with no extension into the cytoplasm. Positive *FOXP3* and CD25 staining occurred within infiltrating interstitial inflammatory T lymphocytic cells, within lymphoid aggregates and cells infiltrating tubules (tubulitis). The staining of the dendritic cell markers, CD83 and DC-LAMP, plasmacytoid DCs, disclosed larger cells with irregularly shaped cytoplasm with positive staining was also observed only scattered among the interstitial cells and in aggregates but not infiltrating tubules.

Localization of Infiltrating Cell Types in Renal Biopsies

Acute Rejection **Stable**

LM

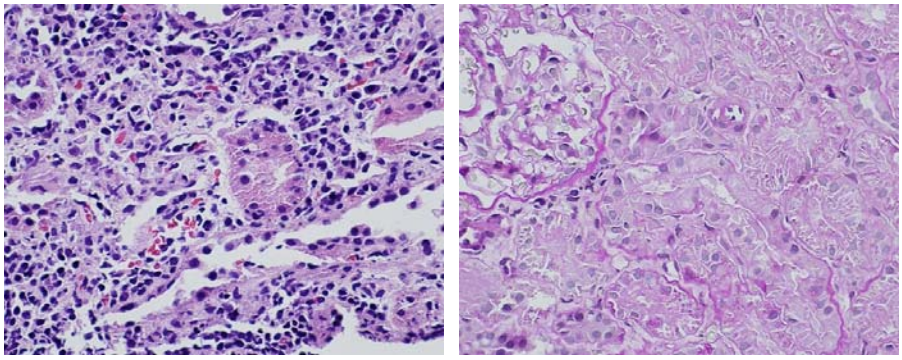

Figure 1: By light microscopy, acute rejection shows acute interstitial inflammation by lymphocytes. No active inflammation is present in stable graft function; H&E and PAS staining; magnification 400x.

CD25

FOXP3

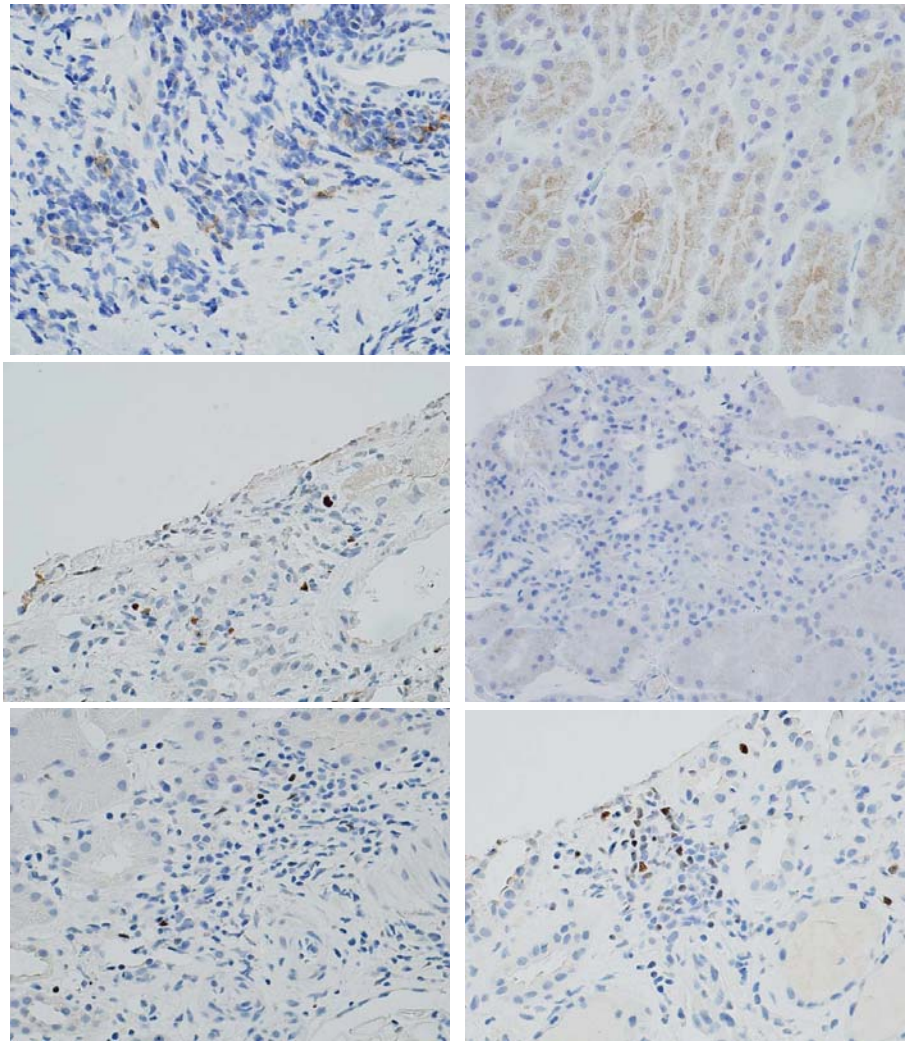

Figure 2: *FOXP3+* and CD25+ T-cells are localized in the interstitium and in infiltrating tubules (tubulitis); dark brown stains; magnification 400x

CD83

DC-LAMP

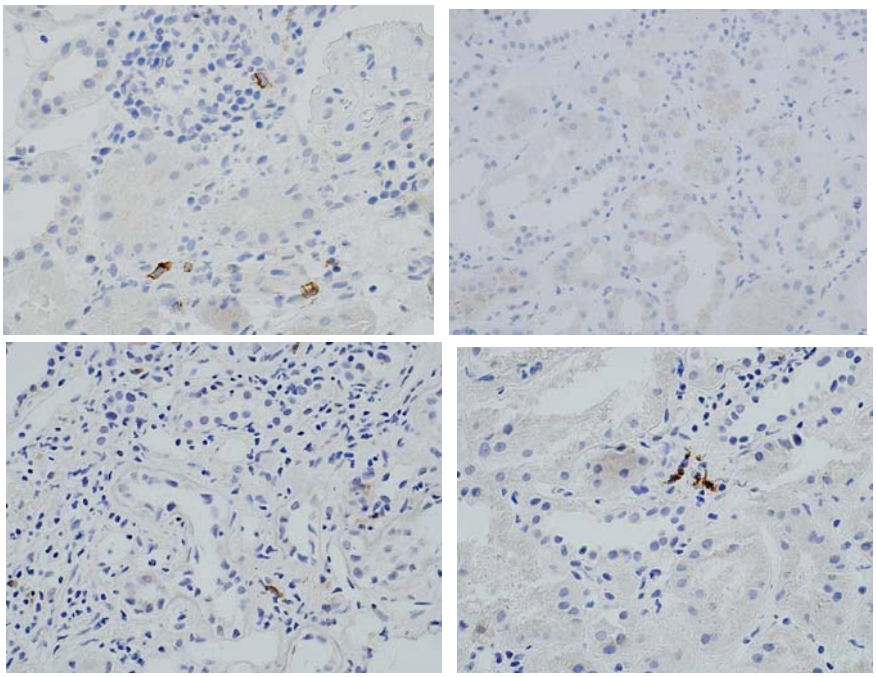

Figure 3. Sparse dendritic cells are localized in the interstitium of renal biopsies. No tubular infiltrating cells are noted; dark brown stains; magnification 400x.

Table I. Quantitation of Infiltrating Cell Types in Renal Biopsies

Acute Rejection (28)	CD 25/ 10HPF	*FOXP3*/ 10HPF	CD83/ 10HPF	DCLamp/ 10HPF
Interstitial	25± 2.5	33.5± 4.2	0.42 ± 0.49	1.85 ± 3.6
Tubulitis Infiltrating tubules	4.1± 0.7	7.8±1.2	-	-
Stable function (22) Interstitial	0.55± 0.3	1.3 ± 0.38	0.3 ± 0.47	0.36 ± 0.48
Infiltrating tubules Tubulitis	0	0.14 ± 0.1	-	-

HPF – high power field

3.1. Statistical Analysis

The number of interstitial CD25+ and *FOXP3*+ lymphocytes cells significantly correlated with tubular CD25+ and *FOXP3*+ cells in acute rejection within the same tissue specimens (Spearman's rank R= 0.62, P=0.0004 and R=0.45 and p< 0.0001 respectively), suggesting a higher interstitial cell infiltration may lead to increased tubular infiltration of the same cells in active inflammation. However, no significant correlation was observed when interstitial and tubular CD25+ cells were compared to interstitial and tubular *FOXP3* cells in acute rejection cases. Although, the reason for this result is not entirely clear, it may depend on the phase of rejection process, individual host response or sampling of the biopsy tissue. Comparison of the interstitial and tubular CD25+ and

FOXP3+ cells in acute rejection to those in stable graft function cases demonstrates significantly higher numbers in acute rejection ($p<0.0001$, Mann-Whitney test) in both locations, for both types of cells.

While the log-transformed *FOXP3*/18S mRNA copies in urinary cells did not correlate with interstitial or tubular *FOXP3+* cells in acute rejection, log CD25/18S in mRNA copies in urinary cells correlated modestly with interstitial CD25 cells ($R= 0.44$, $p=0.02$), despite cells in both tissue locations. Though the mean CD25+ and *FOXP3+* cells were higher in retained grafts than in failed grafts, (*FOXP3* 35$\pm$ 5 vs. 29 $\pm$ 7.6, CD25 27.3 $\pm$ 3.1 vs. 20.5 $\pm$ 3.8), this did not reflect statistical significance.

The cells positive for dendritic cell markers (CD83, DC-LAMP), found only in the interstitial areas, were slightly higher, in acute rejection biopsies (CD83: 0.42 $\pm$ 0.6; DC-LAMP: 1.85 $\pm$ 3.6) than stable functioning grafts (CD83: 0.30 $\pm$ 0.47, DC-LAMP: 0.36 $\pm$ 0.48). No statistical correlations were drawn in comparison with CD25 or *FOXP3* cells by Spearman rank correlation coefficient.

Although aggregates of mononuclear cells were more prominent in all locations (periglomerular, perivenular and arterial) in acute rejection cases, they were assessed for the various cell types in a semiquantitative manner. They were composed of approximately 10% CD25; 0.3% *FOXP3* and less than 1% CD83 and 1-2% DC-LAMP positive cells.

4. Discussion

Significant advances in the field of renal transplantation have improved graft outcomes by introducing new therapeutic options. They are proper donor selection, appropriate preparation of the recipient, early diagnosis of rejection, and powerful

induction and maintenance therapeutic regimen, targeting various immune pathways that result in an adverse allograft response. In addition, non-invasive testing of biomarkers in the urinary cells has shown to be useful, particularly for cytotoxic T cell and other cytokine markers at the mRNA level in predicting the presence of the rejection process in the tissues.

The identification of mRNA of transcription factor FOXP3 in the urinary cells from renal transplant patients has opened a new area in transplant immunobiology towards the study of tolerance (Muthukumar et al. 2005). FOXP3+ cells are regarded as a subset of CD4+ CD25+ T lymphocytic cells, where its expression is commonly induced by TGF-beta (Chen et al 2003). *FOXP3*+ cells are a form of regulatory T cells (T-regs), modulating cell mediated immune reactions. They have been shown to induce tolerance in experimental allografts (Chai et al. 2005). Muthukumar et al demonstrated higher levels of *FOXP3* mRNA in urinary cells from AR patients than those in stable grafts or in chronic allograft nephropathy cases. Moreover, these urinary levels were higher in those who retained their grafts than those who had poor graft function or lost their grafts within 6 months of transplantation (Muthukumar et al. 2005). In the present study, we have set out to confirm these findings, and quantitatively assess the presence and location of *FOXP3*+ T-reg cells in renal biopsy tissues of the same patients from the above study (Muthukumar et al. 2005) with AR and stable graft function.

Based on experimental allograft tolerance (Chen et al. 2003), we hypothesized that stable grafts would harbor increased numbers of *FOXP3* positive T-reg cells in the renal tissue, but lack active tubulitis. Thus, it could be assumed that the *FOXP3* positive cells may fail to enter the urine. While we were able to confirm the presence of increased

CD25+ and *FOXP3+* cells in acute rejection cases in the tissue, both in the interstitium and infiltrating the tubules, resulting in higher urinary *FOXP3* mRNA levels, contrary to our hypothesis, we did not localize a significant number of CD25+ and *FOXP3+* T-reg cells in the tissues of stable functioning allografts ($P<0.0001$). Stable grafts are prone to develop rejection episodes without the presence of *FOXP3+* cells if adequate immuno-suppression is not maintained.

In this study on renal biopsy tissue, significant kappa scores were found with the CD25+ and *FOXP3+* cells in the interstitium and tubules, indicating the relationship between these T-reg cell markers; in AR and stable graft function. However, there was no statistically significant score (CD25 or *FOXP3*) to distinguish those with poor graft function at the tissue level in AR cases.

A network of dendritic cells (DC) have been localized in the kidney in both experimental and normal human kidney (Soos et al. 2006). However, in our study, there was no statistically significant increase in DCs (identified using 2 plasmacytoid DC markers) in AR renal biopsies. This finding suggests that dendritic cell maturation occurs outside the kidney, such as the regional lymph nodes or rarely bone marrow following exposure to soluble antigens or migrating antigen presenting T cells to the lymph nodes. Thus, a higher degree of active dendritic cell participation is not seen in the allograft and may not be considered a target for intervention to modify or attenuate allo-immune reaction in the kidney tissue.

For years, researchers doubted that there were cells that could be specifically responsible for combating immune reactivity. However, recently, immunologists discovered regulatory T cells (T-reg) and their ability to suppress autoimmunity by

impairing activation and proliferation of effector T cells. These cells ensure that immune components refrain from attacking normal tissues. The thymus, where all varieties of T cells mature, eliminates many strong autoreactive cells, and allows T-regs to patrol the body for any destructive immune components.

The mechanisms of how T-regs prevent or modulate auto- or allo-immunity are not fully understood. However, a key step in affecting with autoimmune responses involves signaling interference between antigen presenting cells (APCs) and T cells. By binding to an APC and possibly blocking the receptor site, the T-reg cells may prevent other T cells from attaching to the APC for activation. The T-regs may emit a signal that blocks the APC from sending stimulatory messages to attract other T cells, or it induces the APC to suppress the those cells by releasing cytokines having inhibitory effects. Immunoregulation is a cell contact dependent process and T-regs display a T cell receptor that can bind to a specific antigen. They express T cell markers CD4 and CD25, which are signaled by IL-2 and promote regulatory activities. A crucial role for CD25+CD4+ T-regs in inducing transplantation tolerance is characterized by the expression of the transcription factor, *FOXP3*. This molecular marker is present in high amounts in T-regs, which has the ability to 'quiet' or suppress other T cells and disable autoreactive T cells (Chai et al. 2005).

The findings in this study are novel and can be used to pursue future research in several directions. Perhaps one of the most important will be induction of regulatory T cells in allografts diagnosed with acute rejection following transplantation. *FOXP3*+CD25+CD4+ T-reg cells are naturally present in the normal immune system as a functionally distinct T cell subpopulation. In acutely rejecting transplanted grafts,

naturally arising *FOXP3*+ T-reg cells may be able to induce tolerance, if they are in rich supply. These positive T-reg cells may also help create a T-reg based therapy that could treat and preserve acutely rejecting transplants while limiting the amount of time and quantity of immunosuppressive drugs for patients. Investigative work may be able to produce large numbers of regulatory T cells by transferring the *FOXP3* gene into more prevalent types of T cells. Since they are a beneficial target for designing ways to prevent and treat immunological diseases, immunologists are also trying to identify the molecular events that initiate *FOXP3* production during T regulatory development.

Recently, a new procedure has been considered that would involve removing T-regs from a transplant recipient and culturing them with cells from an organ donor so the T-regs can multiply and strongly suppress the recipient's T cells during the rejection process. Then, these cells can be reintroduced into the patient and the T-regs can combat the recipient T cells, with potential protective effect on the transplanted organ, leading to decreased dependence on immunosuppressive drugs (Chai et al 2003).

Further research for specific molecular markers that can be identified with T-reg cells is essential for their induction or manipulation of to possibly prevent tissue injury. The abundant research into T-regs suggest that this approach is realistic for humans and could be made a viable treatment option to protect transplant recipients. Further studies and clarification of the molecular mechanisms of their development and function, especially the mechanisms of T-reg suppression, are crucial to organ transplantation and may prove to be the ultimate immunologic discovery.

6. Acknowledgments

The above work was conducted at the nephrology and pathology laboratories of

Weill Medical College of Cornell University, New York. This researcher would like to thank Dr. Manikkam Suthanthiran for providing the opportunity to work in his research laboratories and his able guidance in this project, as well as Dr. Darshana Dadhania for all her assistance in supplying clinical data and statistical analysis. She would also wish to thank Dr. Surya V. Seshan for her instruction at every stage of this project and her expertise on Immunopathology and Renal Pathology. She would also extend her gratitude to scientists in Dr. M. Suthanthiran's laboratory such as, Dr. Vijay Sharma, Dr. Ruchuang Ding and Dr. Bagoui Li for devising the molecular probes, generating PCR data and giving assistance with PCR laboratory techniques. This researcher would like to thank Ms. Yifang Liu, Immunopathology technician for her instruction and help in the immunohistochemical procedures. Finally, this researcher would like to thank her teachers Mr. Joseph Sencen, Mr. Michael Inglis, Ms. Kim Dyer and Dr. Ellen Cohen for their support and guidance.

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