

INTRODUCTION

- Diabetes mellitus (DM) is reaching epidemic proportions with an estimated 20.8 million of the US population believed to suffer from type 1, type 2 or other types of DM while a 225% increase is projected between 2000 to 2050 (Engelgau 2004)
- Approximately 30-40% of all kidney transplant recipients in the United States are diabetic before transplantation and 15-20% develop post transplant diabetes mellitus (PTDM) (First 2003, Kasiske 2003)
- Data was obtained from the United Network for Organ Sharing (UNOS), on kidney transplant operations performed between 1996 to 2003 (unpublished communications)
- In total, data from 101,292 kidney transplant recipients showed that 5-year patient survival was 15% and graft survival was 10% lower in patients with type 2 or other/unknown (PTDM) diabetes than non-diabetics (Fig 1A and 1B) (Kaplan-Meier analysis, P<0.0001)

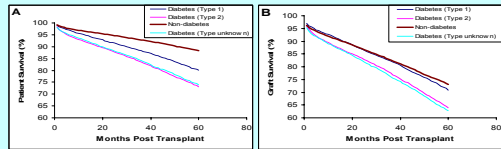


Figure 1A-1B. Comparison of patient (1A) and graft survival (1B) in diabetic kidney transplant recipients (Data from UNOS on 101,292 patients transplanted between 1996-2003)

- Lansang (2006) have compared the incidence of infection (bacterial, viral or fungal) episodes requiring hospitalization between diabetic and non-diabetic patients. The analysis showed the risk of developing such episodes was 43% higher in pre-transplant diabetics and 77% higher in PTDM patients. Septicemia was the most prevalent infection followed by pneumonia, urinary tract infections and others.
- Also diabetics was the leading cause of developing a fungal infection requiring hospitalization (odd ratio=2.4) followed by rejection (odd ratio=1.56) and maintenance tacrolimus (odd ratio=1.53) patient survival in this population.
- It is therefore fundamentally important to recognize diabetic kidney transplant recipients as a subgroup of patients with inferior survival and to explore various methods to improve graft or patients survival.

AIM

To compare various biomarkers of T and B cell activity between diabetic and non-diabetic stable kidney transplant recipients with an aim to optimize post transplant immunosuppressive therapy in these patients.

PATIENTS AND METHODS

- Stable kidney transplant recipients (n=32) were recruited (Table 1).
- 18 patients were diabetic (10 type 1 and 8 type 2) and 14 patients were non diabetic.
- Primary diagnosis of organ failure was diabetic nephropathy in the diabetic group and other etiologies in non diabetic group
- All patients were on triple therapy with mycophenolic acid, prednisone, with either cyclosporine or tacrolimus.

	Diabetic (n=18)	Non diabetic (n=14)
Age (years)	50 ± 13	51 ± 15
Gender (male/female)	14 M/4 F	10 M/4 F
Weight (kg)	84 ± 22	88 ± 25
Time post transplant (months)	43 ± 36	53 ± 33
Cyclosporine/tacrolimus	4/14	7/7

Table 1. Patient demographic characteristics (+/- values are SD)

Printing services provided by the RI-INBRE Centralized Research Core Facility supported by Grant # P20 RR16457 from the NCR/NIH. Research grant from University of Rhode Island Council for Research, Proposal development program is kindly acknowledged.

METHODS

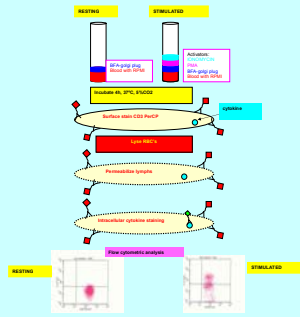


Figure 2. Schematic diagram of intracellular staining method

RESULTS

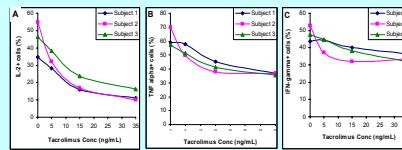


Figure 3A-C. Intracellular cytokine levels in the presence of varying concentrations of tacrolimus in blood samples from three healthy subjects.

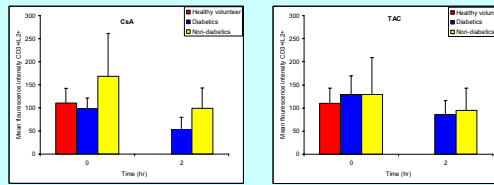


Figure 4. Comparison of IL-2 expression in CD3+ cells in cyclosporine and tacrolimus patients before dose and at 2 hours post dose.

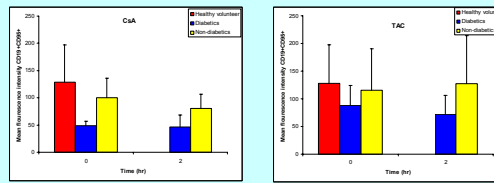


Figure 5. Comparison of CD95 expression on CD19+ cells in cyclosporine and tacrolimus patients before dose and at 2 hours post dose.

- From all transplant recipients, blood samples were obtained prior to the morning dose of immunosuppressive agents (T0) and at two hours post dose (T2).
- The amount of ATP production in PHA stimulated CD4 lymphocyte was measured as an indicator of global immune response using ImmunoKnow™ assay.
- Intracellular production of cytokines interleukin-2 (IL-2), IFN-γ and tumor-necrosis factor-α (TNF-α) in response to mitogenic agents (phorbol-12 myristate acetate/ionomycin) was measured according to an assay adapted from Sindh 2003 (schematic diagram on left) and analyzed using a FACS caliber flow cytometer (BD-Biosciences).
- B-cell activity was investigated by measuring co-stimulatory proteins CD54 (ICAM-1), CD86 (B7.2) and CD95 (Fas antigen) in pokeweed mitogen stimulated CD19+ cells (Berry 2006).

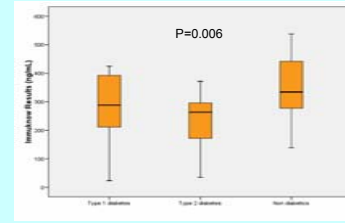


Figure 6. ATP concentration in mitogen stimulated CD4+ cells

ATP level (ng/mL)	Diabetic/Non diabetic
< 225	5 D/2 ND
226-525	12 D/ 11 ND
> 526	1 D/ 1 ND

	IL-2		IFN-γ		TNF-α	
	Rest	Stim	Rest	Stim	Rest	Stim
Healthy	14 ± 2	44 ± 15	16 ± 5	62 ± 62	19 ± 4	580 ± 739
Diabetic transplant recipients (type 1)	14 ± 2	42 ± 21	17 ± 9	175 ± 291	20 ± 4	286 ± 432
Diabetic transplant recipients (type 2)	15 ± 2	32 ± 7*	17 ± 5	339 ± 522	19 ± 3	387 ± 471
Non diabetic transplant recipients	16 ± 2*	62 ± 62	16 ± 5	284 ± 653	23 ± 7*	411 ± 700

Table 2. Median fluorescence intensity of T-cell intracellular cytokines (*P<0.05)

	CD54		CD86		CD95	
	Rest	Stim	Rest	Stim	Rest	Stim
Healthy	85 ± 31	389 ± 285	15 ± 8	39 ± 17	12 ± 12	86 ± 60
Diabetic transplant recipients (type 1)	99 ± 47	323 ± 266	15 ± 8	21 ± 11	12 ± 10	42 ± 25
Diabetic transplant recipients (type 2)	120 ± 42	204 ± 132	18 ± 22	22 ± 18	11 ± 15	37 ± 21
Non diabetic transplant recipients	109 ± 47	335 ± 303	15 ± 10	24 ± 14	12 ± 11	61 ± 58*

Table 3. Median fluorescence intensity of B-cell surface markers (*P<0.05)

CONCLUSIONS

Our results suggests that the concentration of ATP in mitogen stimulate CD4+ cells is statistically lower in both type 1 and 2 diabetic patients versus non diabetics. Expression of intracellular cytokines was lower in diabetic patients only for IL-2 not other cytokines. The results of IL-2 expression in CD3 cells was different based on the type of calcineurin inhibitor used (Csa versus TAC). B cell results showed differences but only CD95 cell surface expression on CD19 cells reached the level of statistical significance. The results of immune marker must be examined in relation to the concentration of various immunosuppressive agents. Adjusting immunosuppressive dosage guided by these markers may prove useful in fine-tuning of immunosuppressive therapy for diabetic patients.

REFERENCES

- Berry V, Magill A, Yost M, Janosky J, Sindh R. Individualizing combination of two antiproliferative immunosuppressants with pharmacodynamic modeling of stimulated lymphocyte responses. *Cytometry A* 2006 Feb;69(2):95-103.
- Engelgau MM, Geiss LS, Saaddine JB, Boyle JP, Benjamin SM, Gregg EW, et al. The evolving diabetes burden in the United States. *Ann Intern Med* 2004 Jun 1;140(11):945-50.
- First MR. Posttransplant diabetes mellitus. *Transplantation* 2003 May 27;75:1769.
- Kasike BL, Snyder JJ, Gilbertson D, Matas AJ. Diabetes mellitus after kidney transplantation in the United States. *Am J Transplant* 2003 Feb;3:178-85.
- Lansang MC, Ma L, Schold JD, Meier-Kriesche HU, Kaplan B. The relationship between diabetes and infectious hospitalizations in renal transplant recipients. *Diabetes Care* 2006 Jul;29(7):1659-60.
- Sindh R, LaVia MF, Paulling E, McMichael J, Burckart G, Shaw S, et al. Stimulated response of peripheral lymphocytes may distinguish cyclosporine effect in renal transplant recipients receiving a cyclosporine+rapamycin regimen. *Transplantation* 2000 Feb 15;69(3):432-6.