DYSREGULATED TH17 CELL IS ASSOCIATED WITH CHRONIC CALCINEURIN INHIBITOR TOXICITY IN RENAL ALLOGRAFT RECIPIENTS

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Introduction

❖ **Calcineurin and its action mechanism**

✓ TCR-MHC interaction results in release of Ca^{2+} from endoplasmic reticulum to cytoplasm, consequently activating calcineurin.

✓ Calcineurin is a, Ca^{2+} Calmodulin dependent Serine/ Threonine phosphatase enzymes.

✓ Calcineurin dephosphorylates, the inhibitory phosphate of NFATc (nuclear factor of activated T cell).

✓ Dephosphorylated NFATc moves to nucleus and initiates IL-2 cytokines transcription.

✓ IL-2 is required for the differentiation, proliferation and survival of T cell.

✓ Calcineurin inhibitor bind to immunophilin protein of T cell. This immunophilin-CNI complex bind to calcineurin and inactivate it, consequently inhibit NFATc dephosphorylation and IL-2 synthesis.

❖ **Th17 (CD4+IL-17+) is a proinflamatory T cell subset**

✓ Develops from naiveTh0 cell in presence of IL-6,TGF-β and transcription factor RORc.

✓ Th17 secrets IL-17 family, IL-21, TNF-α, proinflamatory cytokines.

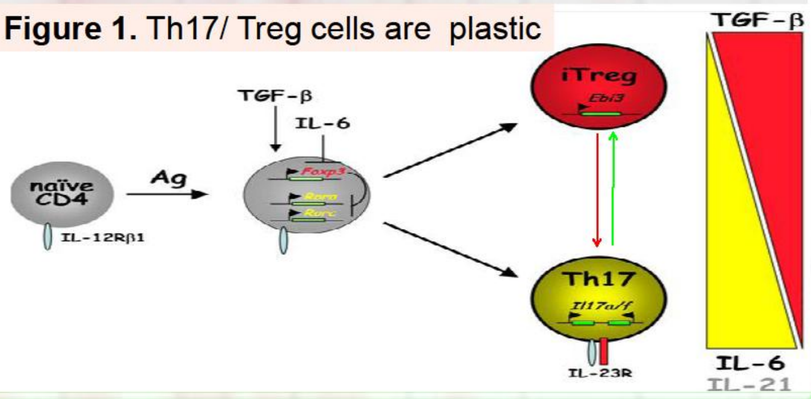
✓ IL-17 induces fibrosis and IL-21 promote Th17, NK, B cell development.

❖ **T reg (CD4+CD25+FoxP3+) is anti inflammatory immunosuppressive cell**

✓ Requires TGF-β for development and secret IL-10,TGF-β and IL-35.

✓ It also suppress other immune cells by contact dependent mechanisms.

Figure 1. Th17/ Treg cells are plastic



Objectives

1. To determine the Th17 and Treg cell frequency in peripheral blood of patients with calcineurin inhibitor toxicity(CNI-T) and stable graft function (SGF).
2. To determine and compare the intragraft expression of mRNA transcript of RORC and FoxP3 in allograft tissue of patient with CNI-T and SGF.

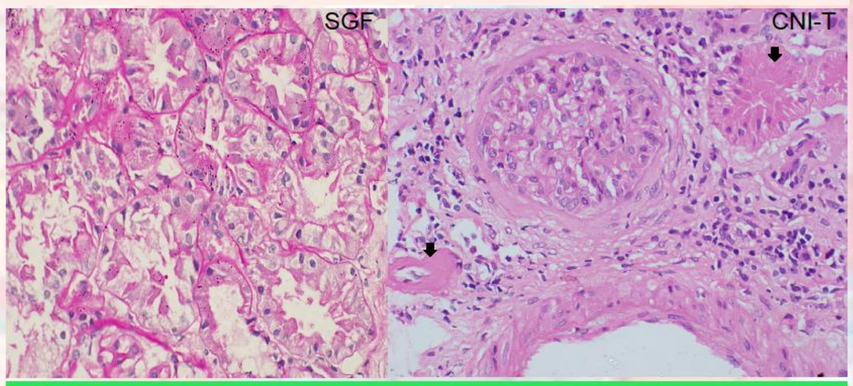
Materials and Methods

Patient recruitment= 38

Stable graft function(10)	CNI Toxicity (28)
✓Normal graft function	✓ Evidence of histological lesion of CNI-toxicity in microvascular compartments on light microscopy
✓No proteineuria	✓Striped fibrosis
✓<25%rise in serum creatinine from baseline i.e stable within previous 6 month	✓Prominent arteriolar hyalinosis
✓<10%Cortical area with tubular atrophy	✓Tubular atrophy lesions.
	✓No evidence of pyelonephritis, Viral nephropathy, Recurrence of native kidney disease .

Materials and methods

Figure 2. Histology of renal allograft of patient with stable graft function and CNI-T



Circulating Th17 and Treg cell frequency analysis

Collect veinous blood in heparinised vials

Frequency analysis of T cell subpopulation

Th17 $CD4^+IL\text{-}17A^+$ → Stimulated with PMA, Ionomycin and Monensin for 5h

T regs $CD4^+CD25^+FoxP3^+$ → Surface staining for CD4-FITC, CD25-PE, conjugated mouse monoclonal antihuman Abs

RBCs were lysed with BD FACS lysing solution

Fixation and permeabilisation with Cytofix/Cytoperm kit for (Th17) and with BD Human FoxP3 Buffer set for(Tregs)

Intracellular staining for IL-17 A –PE and FoxP3-Alexaflour-647 conjugated mouse monoclonal Abs

Acquired on Flow Cytometry (BD FACS Calibur)
Data was analyzed with FCS express 5 software

Analysis of intragraft mRNA transcript expression of RORC and FoxP3

✓Collected allograft biopsy in Trizole
✓Isolated total RNA by Trizole methods
✓500ng RNA was converted in to c-DNA, by using superscript II reverse transcriptase enzymes (Invitrogen Kit)
✓2µl of cDNA was used for Taqman real time PCR gene expression study on ABI-7500 machine
✓Fold change was calculated by 2-^^ct method

Results

Table-1. Patients characteristics

Patients characteristics	SGF	CNI-T	P value
Patients age (Years)	46.25±15.4	51.0±31.70	0.270
Post Tx biopsy duration (months)	70.01±15.4	51.0±31.70	0.058
Baseline creatinine(mg/dl)	1.22±0.41	1.12±0.297	0.597
Serum creatinine at biopsy (mg/dl)	1.44±0.33	2.30±0.86	0.068
Urine protein/24hrs (gm)	0.28±0.084	2.41±2.13	0.073
Donor age(Years)	55.0±2.16	51.90±10.38	0.574
eGFR (mL /min/1.73 m^2)	60.75±17.0	35.85±15.67	0.038
Tac level (ng /ml)	4.62±0.45	5.05±1.38	0.335
HLA mismatch score	2.7±0.823	2.92±0.716	0.548

Results

Table-2. Frequency of circulating Th17 and Treg cell

Characteristics	SGF	CNI-T	P value
CD4 Th cell %	31.91±2.95	26.89±7.05	0.037
Th17 cell (CD4+IL-17A) %	12.20± 2.16	20.24±.3.7	<0.001
T reg cell (CD4+CD25+FoxP3) %	5.03± 1.16	2.73± 1.15	<0.001
Th17/ Treg cell ratio%	2.55±0.753	8.66±3.30	<0.001

Figure 3. Representative flow cytometry image panel of (A).CD4, (B).Th17 and (C).T reg cell.

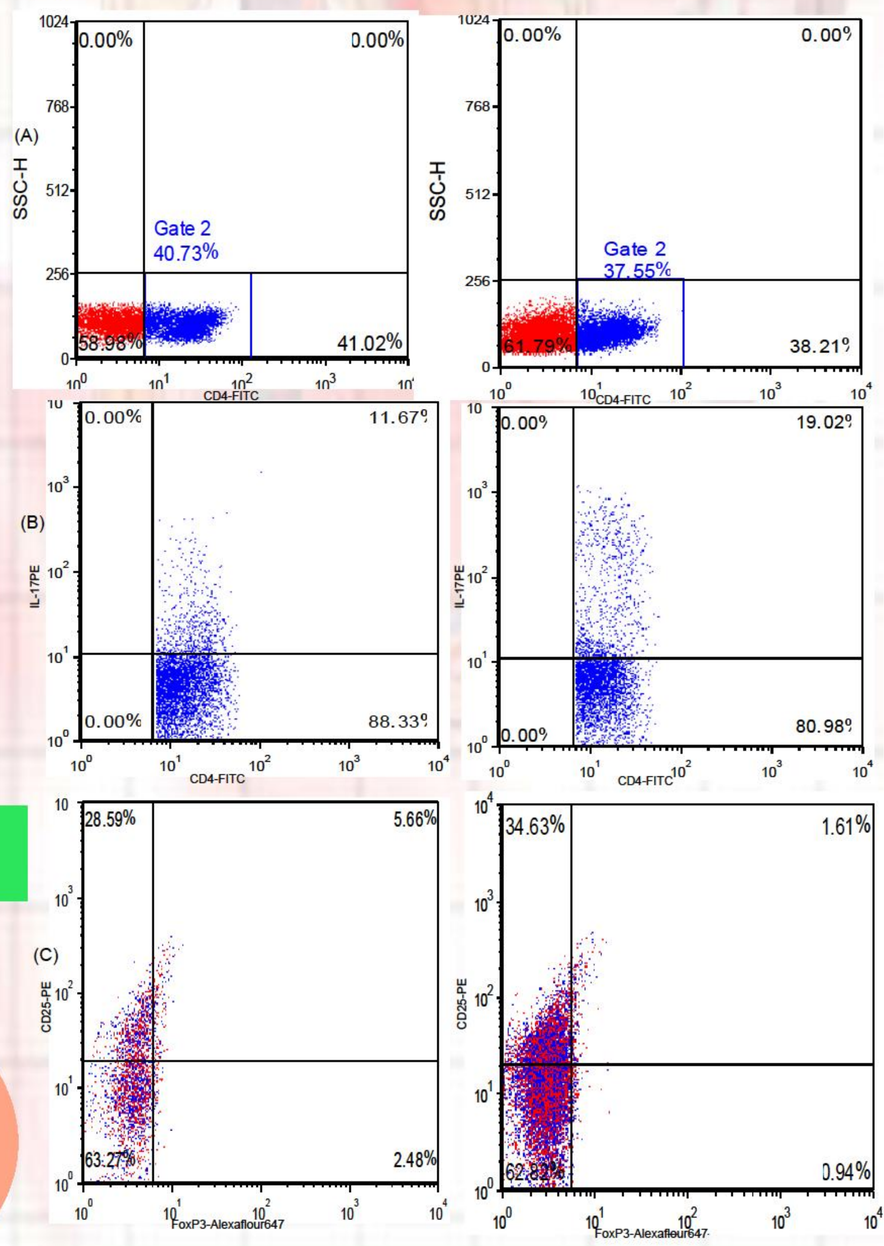
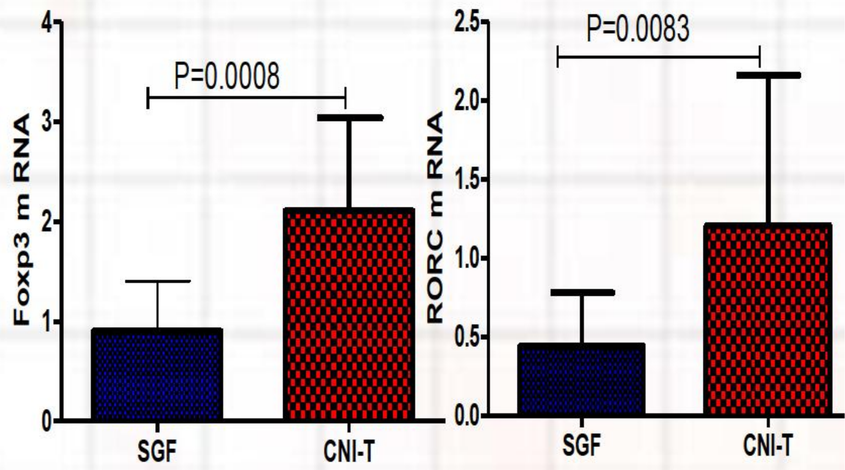


Figure4. Intragraft m-RNA transcript expression of RORc (Th17) and FoxP3 (Treg)



Conclusions

✓Th17 cell remains dysregulated in chronic renal allograft recipient patients with calcineurin inhibitor toxicity.

✓ Frequency of circulating Th17 cell remains high in blood as well as in intragraft tissue of patients with CNI-T.

✓Frequency of T reg cell decreases in circulating blood as well as in intragraft tissue of patients.

✓Chronic allograft injury may be due to Th17 cell which might play a role in stripped renal fibrosis, characteristic of chronic CNI-T.

Acknowledgments

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