INCREASED INTRAGRAFT mRNA TRANSCRIPT OF GRANZYME-B IS ASSOCIATED WITH CHRONIC TRANSPLANT GLOMERULOPATHY

SP-779

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Introduction

- Chronic transplant glomerulopathy(CTG) and interstitial fibrosis and Tubular atrophy(IF/TA) are two major cause for graft loss in long time after transplant.
- Up to 20% transplant patient loss graft due to of transplant glomerulopathy alone.
- CTG is associated with proteineuria, C4d deposition in peritubular capillary and donor specific antibody against the either or both class of HLA.
- Interstitial fibrosis and tubular atrophy (IF/TA) is associated with fibrosis without any specified lesion

Cytotoxic T cell

- Cytotoxic T cell is, Granzyme-B positive CD3+CD8+ T cell.
- On activation it secrets serine protease Granzyme B, Perforin, lymphotoxin and express surface receptor Fas L.
- Perforin form pore in kidney cell and disturb osmolitic balance of cell.
- Granzyme B, a serine protease, cleaves procaspase in to active caspase and induce apoptosis in kidney cell.
- Granzyme-B also cleave cytoskeleton protein, metalloproteinase and induce apoptosis in endothelial cells..
- FasL binds to FasR of kidney cell and induce apoptosis in them..
- The exact mechanism of immune injury in such conditions are not well established.

AIM

- To study the frequency of peripheral blood cytotoxic T cell in SGF, IF/TA and CTG patients.
- To determine the Granzyme B mRNA expression in allograft biopsy tissue in these conditions

Material and Methods

Patient recruitment (Total -34)

CTG-(13)
- ✓Nephrotic range Proteineuria
- ✓C4d + in peritubular capillary
- ✓DSA positive

IF/TA (N=14)
- ✓Patchy fibrosis
- ✓No evidence of BK virus nephropathy
- ✓Chronic UTI,
- ✓Pyelonephritis
- ✓Recurrence of basic disease

SGF-(7)
- ✓Normal graft function with rise in serum creatinine which is stable within previous six months

All patients were recruited as per criteria of Banff-2007

Materials and Methods

Frequency analysis of Cytotoxic T cell

Collect peripheral veinous blood sample in heparin vials

Stimulated cells with PMA, Ionomycin and Monensin for 5h at 37C

Done surface staining with PerCpCy5.5 labeled mouse antihuman CD3 and APC conjugated mouse anti human CD8 Abs

Lysed RBC, with BD FACS RBC lysing solution

Fixed and Permeabilized cell with Cytofix/Cytoperm solution

Done intracellular staining with PE conjugated mouse antihuman Granzyme B Abs

Acquired cell on Flow Cytometry machine (BD FACs calibur)

Gene expression analysis of Granzyme B

Collected 1 core biopsy in Trizole and stored in LN2

Isolated RNA with graft biopsy tissue using Qiagen RNA mini kit

500ng RNA was converted in to c-DNA by using MMLV superscript II Reverse Transcriptase (RT)

2ul c-DNA was used in 20µl reaction volume for Granzyme-B m RNA expression determination

Used unlabelled Primer and Fam labeled MGB Probe with non fluorescent quencher

Run Real Time PCR ABI -7500 Machine)

Statistical analysis:

- ✓The means values in different groups were compared with one way analysis of variance test.
- ✓The relative change in Granzyme B mRNA expression was calculated by 2-^^Ct method
- ✓p<0.05, is considered to be significant,
- ✓ Data analysis was done using SPSS 18.0 software.

Results

Table 1 | Demographic and clinical characteristics of patient

Characteristic	SGF	IF/TA	CTG	P value
Pt. age (Years)	46.28±8.65	36.50±8.34	41.8±12.7	0.201
Baseline creatinine	1.22±0.41	1.12±0.29	1.17±0.39	0.868
Serum creatinine (mg/dl)	1.41±0.33	2.30±0.86	2.38±0.84	0.120
Urine protein (gm/24hrs)	0.20±0.13	1.22±0.96	2.67±0.73	0.027
Tacrolimus level	4.62±0.45	5.05±1.38	4.80±1.60	0.336
eGFR	60.75±17.01	35.85±15.67	37.15±17.31	0.038
Biopsy after Tx (Months)	70.00±15.42	51.71±3.06	63.92±41.00	0.542

Table 2. Mean % of CD3+CD8+T cell and CD3+CD8+ Gzm B+ in SGF, IF/TA and CTG group respectively.

Characteristic	SGF	IF/TA	CTG	P Value
CD3%	66.56±6.34	62.10±11.71	58.88±8.75	0.392
CD8%	13.20±1.02	17.39±5.78	17.20±6.61	0.436
CTLc%	26.35±2.95	16.44±4.09	12.62±1.68	<0.001

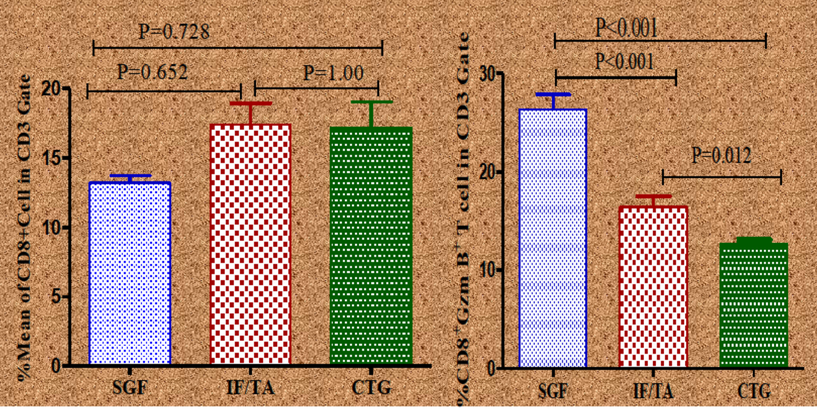


Figure 1. Bar-diagram shows no significant difference between the groups for the CD3+CD8+ T cell.
While for CD3+CD8+ Gzm-B +T cell was significantly low in CTG compared with IF/TA and SGF .

Results

Figure 2. Relative fluorescence intensity (RFI) in one of the representative sample from a patient with CTG

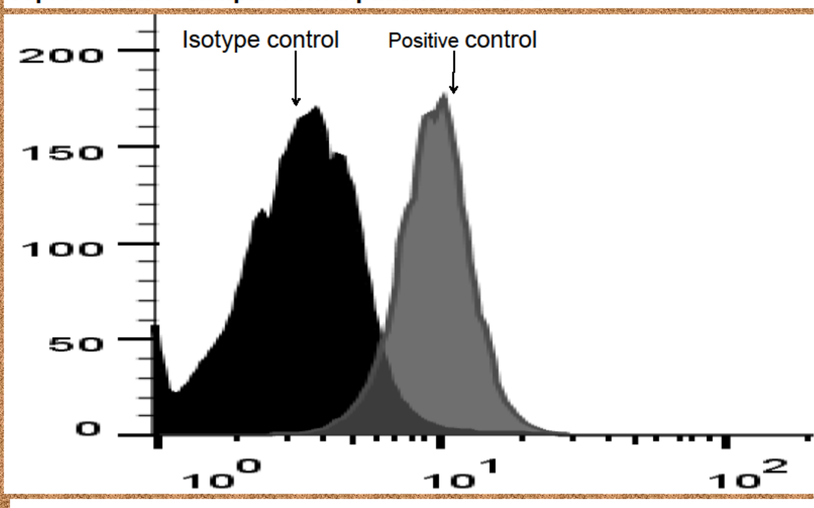
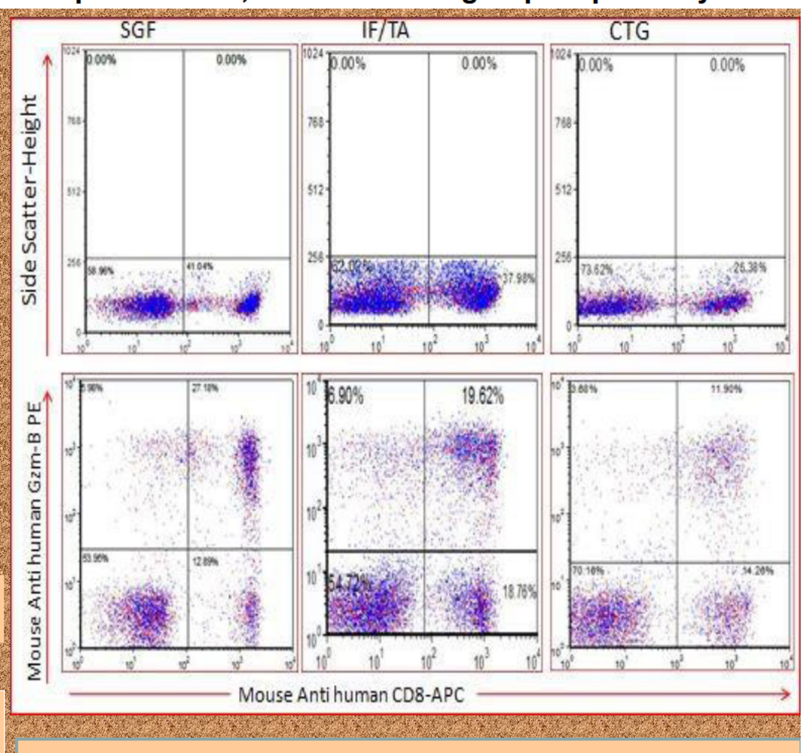
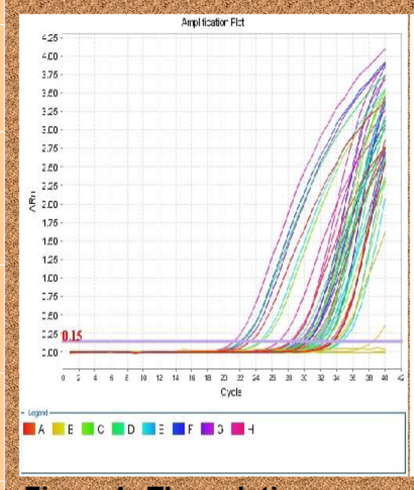


Figure3. Representative flowcytometric picture of renal transplant in SGF, IF/TA and CTG group respectively



Granzyme-B mRNA expression in allograft biopsy in SGF, IF/TA and CTG group



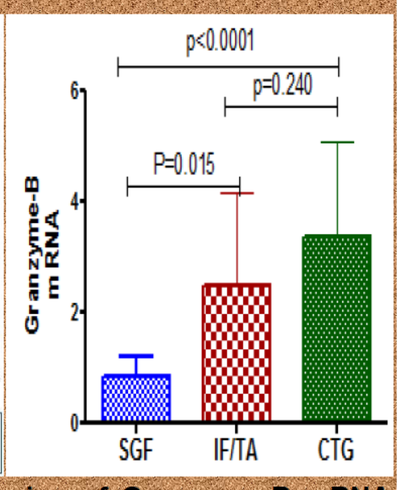


Figure4. The relative expression of Granzyme B mRNA was significantly high in IF/TA and CTG group compare to stable graft function.
Compare to SGF fold expression in IFTA was (2.44) and in CTG (3.35).

Conclusions

- ✓Low frequency of cytotoxic T cell in peripheral blood and increased Granzyme-B m-RNA expression in intragraft tissue is associated with CTG and IF/TA.
- ✓ Low frequency of cytotoxic T cell may be due to of either release of Granzyme –B in serum or sequestration of cytotoxic T cells in graft tissue .
- ✓Low peripheral blood cytotoxic T cell in such clinical scenario may be peripheral blood signature of these conditions

Acknowledgments:

Brijesh Yadav is a "DST-INSPIRE Fellow" supported by Department of Science and Technology, New Delhi, India.

DOI: 10.3252/pso.eu.52era.2015
ePosters supported by F. Hoffmann- La Roche Ltd.