Measurement of Immune Function and Alloresponse in Kidney Transplant Recipients with Cancer

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OBJECTIVES

Immunosuppression increases the risk of cancer in Kidney Transplant Recipients (KTR) these malignancies have separate immune suppressive effects. Delineating the specific immune defects in KTR with cancer has the potential to guide immunosuppression management.

Using a cross sectional design we measured and compared donor specific antibodies (DSA), alloreactive effector and regulatory T cell (Treg) immunity, and natural killer (NK) cell function

METHODS

Donor specific antibodies (DSA), alloreactive effector and regulatory T cell (Treg) immunity, and natural killer (NK) cell function was measured in KTR with (n=29) and without (n=17) cancer.

Patient blood was stained with fluorochrome conjugated antibodies for markers of memory B cells, CD8+ γδ T cells and NK cells and Regulatory T cells. Lifecodes Tepnel ID, LSA1 and LSA2 screen kits (Immucor, USA) were used to determine antigen specific anti-HLA. KTR allo reactive T cells were measure by MLR, which consisted of $3x10^5$ KTR PBMCs plated on an anti IFN-γ antibody coated ELISPOT plate, co-incubated with B cells lines at $5x10^4$/per B cell line for 24h. KTR PBMC $3x10^6$/ml PBMC were co-cultured with $1.5x10^4$ (20:1) K562 cells in triplicate, for 6h and Lactate dehydrogenase (LDH) release measured. KTR Tregs were co-incubated with CD4+CD25- effector T cells in the presence of anti-CD3/CD28 expander beads (Invitrogen, USA) at a bead to cell ratio (1:4) for 8-9h. Suppression was measure by CD154 expression and standard CSFE dilution assays.

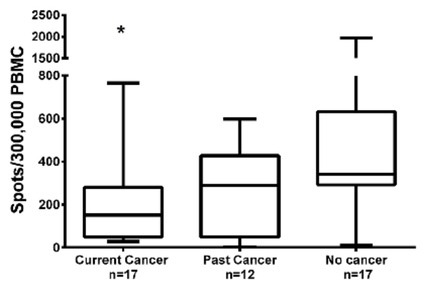

Figure 1: Panel of Reactive T cell (PRT) Interferon-γ ELISPOT assay.

Box and whisker plot depicting Interferon-γ release in response to B cell allo-antigen presentation from KTR with current cancer, past cancer and no cancer. KTR with cancer had significantly lower alloresponse compared to KTR with no cancer, Kruskal-Wallis p-value = 0.031

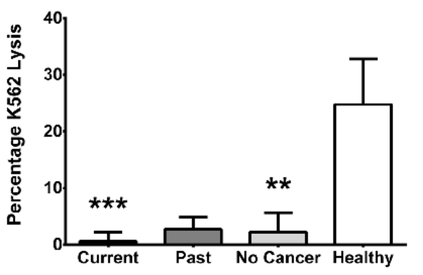

Figure 2: Natural Killer (NK) cell function as measured by Lactate Dehydrogenase (LDH) release.

Column graphs depicting the accumulative data of Lactate Dehydrogenase (LDH) release healthy (no fill, n=5), KTR with no cancer (no cancer (light grey), n=15) and 6 KTR who has had cancer in the past (past (dark grey), n=7) and KTR with current cancer (current (black), n=10) respectively. Both KTR with current cancer and those with no cancer have significantly lower LDH release than healthy controls.

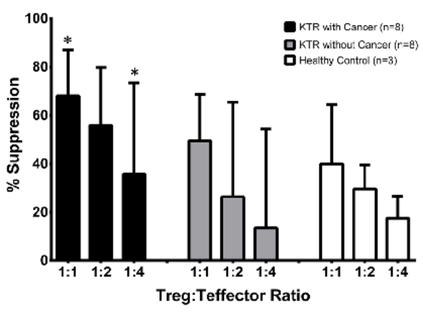

Figure 3: Isolated peripheral blood regulatory T cell suppression of CD154/CD40L.

A column graph showing the accumulative mean ± standard deviation of KTR with cancer (n=8, Black), without cancer (n=8, Grey) and healthy control Tregs (n=3, White) at titrating Treg:Teff ratios, 1:1, 1:2 and 1:4.

RESULTS

Immunosuppressive drugs, doses and serum levels did not differ between KTR with and without malignancies. The prevalence of DSA did not differ between KTR groups (16-26%, p=0.661).

The frequency of alloreactive-and mitogen-induced IFN-γ producers by ELISPOT was significantly lower in KTR with cancer (p=0.008 and 0.019 respectively).

We also observed diminished NK cell function median (range) 0 (0-5.5) vs. 1.6 (0-10.5), (p=0.037), as well as a higher frequency of Treg with more potent suppressive Treg capacity in KTR with cancer compared to KTR withouth cancer: median (range), 36 (13-73) vs. 13 (5-54), (p=0.015).

CONCLUSIONS

Together the findings indicate complex and multifaceted immune defects in KTR with cancer that cannot be attributed to immune suppression alone. Our data suggest that delineating the specific functional and phenotypic immunological fingerprint in KTR has the potential to individually guide manipulation tailored to the transplant recipient's immune status.

REFERENCES:

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