Changes in B cell phenotypes in peripheral blood of patients with long-surviving renal transplant.

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INTRODUCTION

Studies of peripheral blood B cells subsets in renal transplant patients with operational tolerance off immunosuppressive therapy have identified several differences in B cell subsets.[1-4] Here we studied B cells in blood lymphocytes from long-surviving renal transplant patients on immunosuppression. Populations of $CD4^+$ T cells, $CD8^+$ T cells, NK cells and B cells, along with subpopulations of B cells, in long-surviving renal transplant recipients were compared to healthy volunteers.

AIMS

This study aimed to examine changes in B cell subsets in Healthy volunteers (HV), renal patients on dialysis and patients that received kidney transplants at various time points, which may indicate transplant tolerance and potentially identify patients who could reduce immunosuppressive medication.

METHODS

Fresh blood was taken from healthy volunteers (HV) (n=44) and stable renal transplant patients with grafts surviving >10yrs (RT) (n=25). Whole blood was examined for T, B, and NK cells (CD3/CD4/CD8/CD45/CD19/CD16&CD56). Peripheral blood mononuclear cells (PBMCs) were isolated from the remaining blood and stained with a panel of monoclonal antibodies (CD19/CD21/CD24/CD27/CD38/CD45/IgD/IgM) to identify B cell subpopulations.

Table 1. *B cell subset marker definition*

B cell subsets	Markers
Total B cells	$CD45^+CD19^+$
CD21hi B cells	$CD45^+CD19^+CD21^+$
CD21lo B cells	$CD45^+CD19^+CD21^{-/lo}$
Naïve B cells	$CD45^+CD19^+CD27^-IgD^+IgM^+$
Memory B cells	$CD45^+CD19^+CD27^+$
Switched Memory B cells	$CD45^+CD19^+CD27^+IgD^-IgM^-$
Class Unswitched B cells	$CD45^+CD19^+$ $CD27^+IgD^+IgM^+CD38^-$
Marginal Zone B cells	$CD45^+CD19^+CD27^-IgD^+$
Transitional B cells	$CD45^+CD19^+CD27^+IgD^+IgM^+CD38^+CD24^+$
Plasmablast	$CD45^+CD19^+CD27^+IgD^-IgM^-CD38^+$

Data was acquired on BD FACSCanto II using BD FACSDiva software (v8.0) and analysed using FlowJo.

Table 2. *Demographics of healthy volunteers (HV) and transplant patients with grafts surviving >10yrs (RT)*

	HV (n=14)	RT (n=25)
Male/Female	7/7	16/9
Mean age	48.71 (±13.55) years	58.68 (±11.63) years
Average yrs post-transplant	-	16.22
Pre-Tx immunosuppression	-	Steroids: 1 Cyclophosphamide: 1 Rituximab /other: 0
Current immunosuppression	-	Tacrolimus: 10 Cyclosporine: 4 Sirolimus: 1 Everolimus: 6 Azathioprine: 3 Mycophenolate Mofetil (MMF): 20 Prednisone: 20

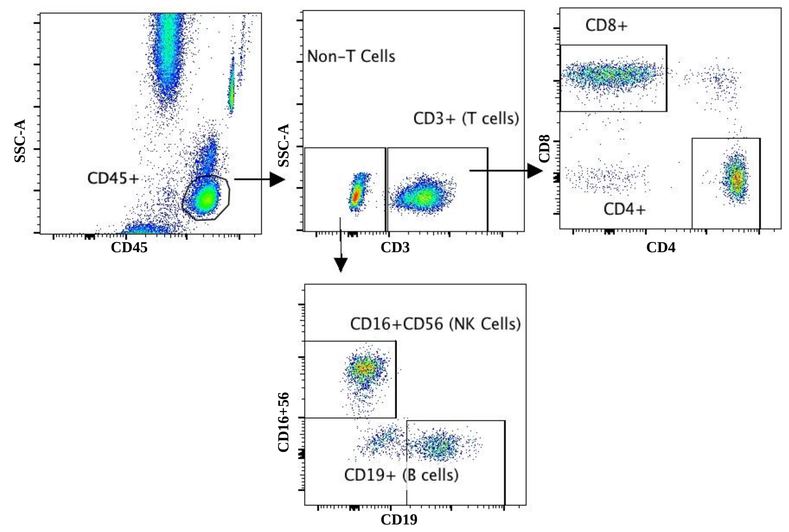


Fig 1. *Gating strategy for major lymphocyte subset analysis for T, B and NK cells. Lymphocyte populations were examined after FSC vs SSC gating and doublets exclusion.*

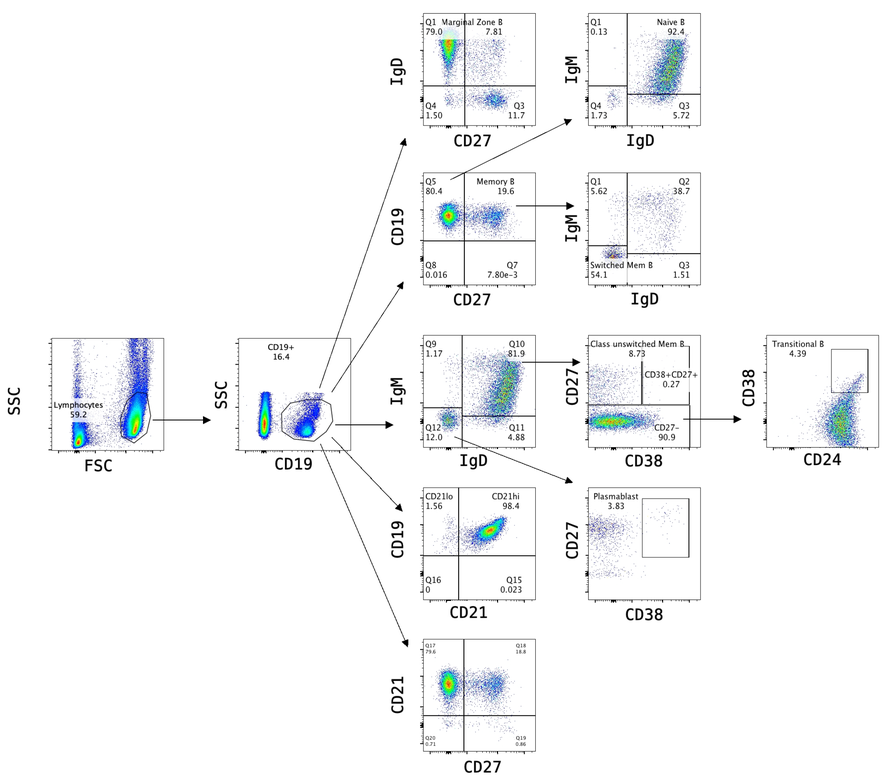


Fig 2. *Gating strategy for B cell subset analysis. Lymphocyte populations were examined after FSC vs SSC gating and doublets exclusion before gating on $CD19^+$ cells for total B cells. B cells were further examined for subsets.*

RESULTS

Analysis for T, B and NK cells

1. Comparison of Dialysis patients and RT with HV

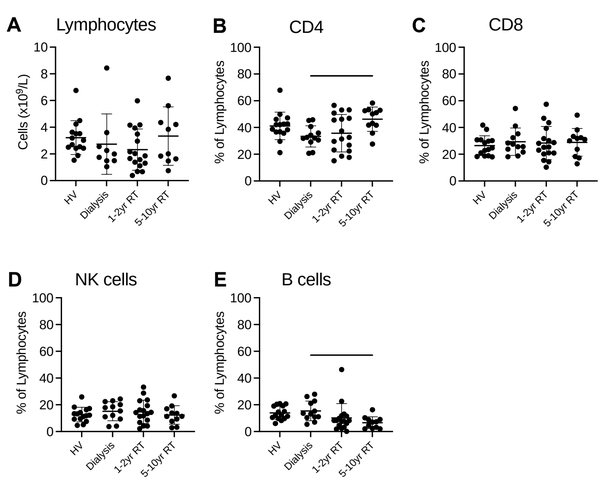
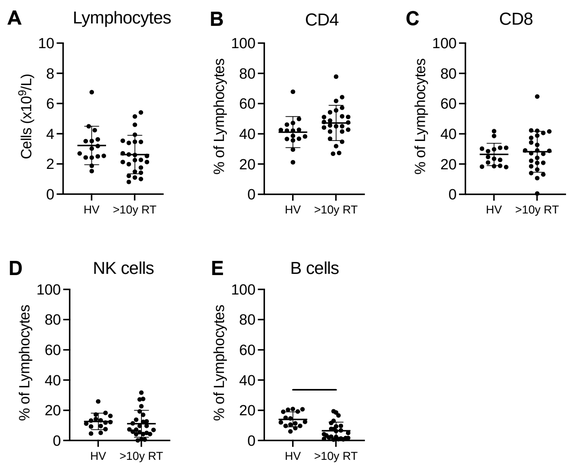


Fig 3. 1. *Analysis of T, B and NK cells in fresh blood of HV and patients on Dialysis, RT 1-2 years and 5-10 yrs post transplant (upper panel) and **2.** HV compared to RT >10yrs post transplant. Lymphocyte counts were not different in 5 groups. B cells were reduced in RT 5-10yrs and >10 yrs compared to HV. $CD4^+$ cells were less in patients on Dialysis compared to those in RT 5-10yrs post transplant.*

2. Comparison of >10yrs RT with HV



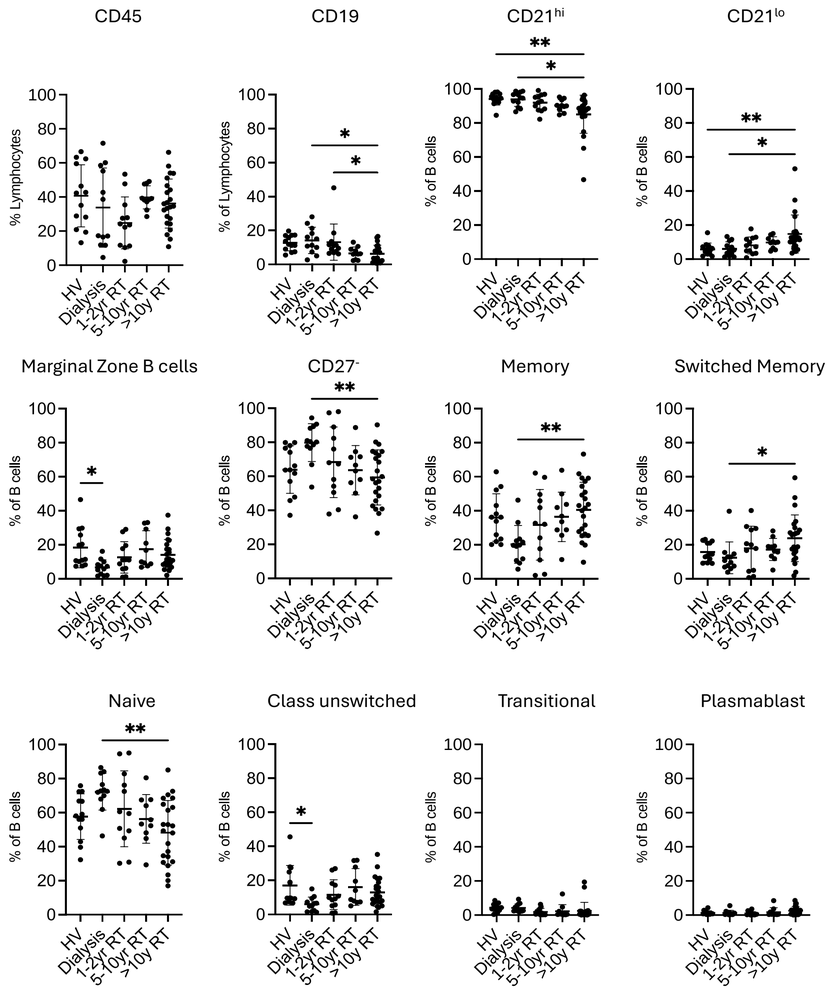


Fig 4. *Analysis of B cells subsets in fresh blood of HV and patients on Dialysis, RT 1-2 years , 5-10 yrs and >10 yrs post transplant. $CD19^+$ B cells were lower in RT>10 yrs compared to those on dialysis and patients with 1-2yrs post transplant. Naïve B cells, memory cells and switched memory B cells. Dialysis patients had lower Marginal zone B cells and class unswitched memory B cells. $CD21^{hi}$ B cells were lower in RT>10yrs compared to Dialysis patients and HV and conversely $CD21^{lo}$ cells were higher in RT>10yrs compared to those two groups.*

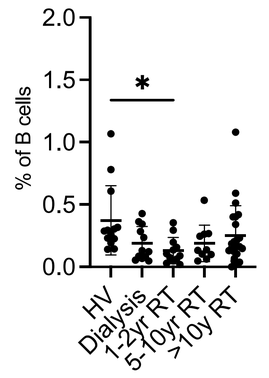


Fig 5. *Comparison of B regulatory cells (Breg) in patients on dialysis, RT 1-2yrs, 5-10yrs and >20yrs post-transplant with HV. Bregs are identified as $CD19^+CD38^{hi}CD24^+CD27^+$. 1-2yr RT has significantly lower B reg compared to HV ($p<0.05$)*

CONCLUSIONS

- Lymphocyte counts were not different in patients on dialysis and RT patients compared to HV.
- There were no difference in CD8 and NK cells in different groups.
- CD4 cells were lower in dialysis patients compared to RT>10yrs.
- B cells were significantly lower in RT >10yrs compared to patients on dialysis and RT 1-2 yrs. post-transplant.
- Naïve B cells were lower in RT>10yrs compared to patients on dialysis.
- Memory B cells and switched memory B cells were higher in RT>10yrs compared to patients on dialysis.
- Both Marginal zone B cells and class unswitched Memory B cells were significantly lower in patients n dialysis compared to HV.
- Ratio of activated Treg to B cells was higher in RT >10 yrs compared to HV. This higher proportion of Treg to B cells may be clinically relevant and potentially demonstrate increased immunological suppression in long-surviving renal transplant recipients.

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