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Sirolimus Increases T-Cell Abundance in the Sun Exposed Skin of Kidney Transplant Recipients

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Background. Kidney transplant recipients (KTRs) receiving the mammalian target of rapamycin inhibitor sirolimus may display a reduced risk of skin cancer development compared to KTRs receiving calcineurin inhibitors. Despite studies investigating the effects of these 2 drug classes on T cells in patient blood, the effect these drugs may have in patient skin is not yet known. **Methods.** Fifteen patients with chronic kidney disease (not recipients of immunosuppressive drugs), and 30 KTRs (15 receiving a calcineurin inhibitor, and 15 receiving sirolimus) provided matched samples of blood, sun exposed (SE) and non-SE skin. The abundance of total CD8⁺ and CD4⁺ T cells, memory CD8⁺ and CD4⁺ T cells, and regulatory T (Treg) cells in each sample was then assessed by flow cytometry. **Results.** Sirolimus treatment significantly increased absolute numbers of CD4⁺ T cells, memory CD8⁺ and CD4⁺ T cells, and Treg cells in SE skin versus paired samples of non-SE skin. No differences were found in the absolute number of any T cell subset in the blood. Correlation analysis revealed that the percentage of T cell subsets in the blood does not always accurately reflect the percentage of T-cell subsets in the skin of KTRs. Furthermore, sirolimus significantly disrupts the balance of memory CD4⁺ T cells in the skin after chronic sun exposure. **Conclusions.** This study demonstrated that immunosuppressive drug class and sun exposure modify the abundance of multiple T-cell subsets in the skin of KTRs. Correlation analysis revealed that the prevalence of Treg cells in KTR blood does not accurately reflect the prevalence of Treg cells in KTR skin.

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Kidney transplant recipients (KTRs) experience up to a 100-fold increased risk of nonmelanoma skin cancer compared to the general population.¹ The use of immunosuppressive drugs, which are essential for long-term renal

allograft survival, is complicated by an increased risk of malignancy. Contributing factors are thought to include the inhibition of regulatory pathways important in cellular senescence² and reduced immune-mediated clearance of

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malignant cells.³ Much interest has focused on whether the increased risk of skin cancer in transplant recipients is due to effects of immunosuppressive drugs on specific immune cell populations. Calcineurin inhibitors (CNI), such as tacrolimus and cyclosporine, and mammalian target of rapamycin inhibitors (mTORi), such as sirolimus (SRL), have been described to have differential effects on the abundance of circulating regulatory T (Treg) cells in patients^{4,5} as well as circulating memory CD8 T cells in mice.⁶ Furthermore, the immune phenotype in the blood may be predictive of the risk of cutaneous squamous cell carcinoma development after kidney transplantation.⁷

mTORi have both antineoplastic and immunosuppressive properties. Randomized controlled trials in KTRs have shown the use of SRL, compared with CNI, is associated with the development of fewer de novo cutaneous squamous cell carcinomas⁸ and an increased time to first skin cancer development.⁹ SRL treatment has been shown to increase numbers of circulating forkhead box P3 (FOXP3⁺) Treg cell¹⁰ and memory CD8 T-cell⁶ populations. Differential effects of mTORi and CNI on Treg cells and memory CD8 T-cell populations in the skin may be expected to contribute to the differential skin cancer risk, yet although previous research has examined the effects of immunosuppressive drugs on immune phenotypes in the peripheral blood, very few studies have examined corresponding changes in skin; the site where cancer most frequently develops in these patients. Much of our understanding of the interactions and function of memory CD8⁺ T cells is obtained from mouse studies however the pathogenic process causing skin cancer development may be different in humans with exposure to ultraviolet (UV) light occurring over many years.

Ideally, the assessment of peripheral blood immune cell populations could be used as a marker of immune phenotype in the skin and other peripheral tissues. However, whether immune cell subtypes in the blood of KTRs are representative of that found in the skin remains uncertain. In this study, we examined T-cell populations in peripheral blood, sun exposed (SE), and non-SE skin biopsies derived from chronic kidney disease (CKD) patients who were not receiving immunosuppressant medicines, and compared these findings to those derived from individual KTRs receiving either SRL or a CNI, to define whether immune phenotype in the skin can be predicted from peripheral blood analysis in these patient cohorts. We also studied the differences in T cell populations between the different immunosuppressants and if this was altered by sun exposure.

MATERIALS AND METHODS

The study protocol was approved by the Metro South Human Research Ethics Committee (HREC/14/QPAH/513), and all patients who participated in the study provided written informed consent. The study was performed in accordance with Strengthening the Reporting of Observational Studies and Epidemiology guidelines.¹¹

Patients and Study Design

Forty-five ambulatory participants were recruited from the Princess Alexandra Hospital (Brisbane, Australia) renal department including 15 KTRs receiving SRL, 15 KTRs receiving a CNI (either tacrolimus or cyclosporine) and 15 CKD patients not receiving immunosuppressive therapy. Patients

were 18 years or older and of white ethnicity. Eligible KTRs also received treatment with any combination of azathioprine, mycophenolic acid, and prednisone. Exclusion criteria included transplantation within the past 12 months, pregnancy, receipt of T or B cell-depleting antibodies within the past year, previous application of topical antiskin cancer therapies to the planned biopsied arm, suspected or proven infection within the past fortnight, current nonskin malignancy or systemic chemotherapy for the treatment of malignancies in the previous 12 months.

Blood Collection and Skin Biopsy

Two skin biopsies were taken from the left or right arm per patient preference and the avoidance of dialysis vascular access. The skin was prepared with povidone iodine and xylocaine with adrenaline was used for local anaesthesia. A 4-mm punch biopsy was obtained from the medial aspect of the upper arm and the dorsal aspect of the forearm, representing non-SE (non-photodamaged) and SE (photodamaged) skin, respectively. Care was taken to ensure that no macroscopically evident premalignant or malignant skin lesions were biopsied. The skin biopsy samples were collected in normal saline. Ten milliliters of blood were collected by venesection from the cubital fossa into an ethylenediaminetetraacetic acid vacuum tube, and all samples were kept on ice and processed within 2 hours.

Antibodies and Reagents

Antibodies and reagents used for sample analysis by flow cytometry included: Human Fc receptor binding inhibitor (eBioscience, San Diego, CA), anti-CD45RA FITC (HII100; Biologend, San Diego, CA), anti-CD4 AF700 (OKT4; eBioscience), anti-CD8a APC-Cy7 (HIT8a; Biologend), anti-TCR α/β PE (WT31; eBioscience), anti-CD45RO PerCP/Cy5.5 (UCHL1; Biologend), anti-CD25 APC (Bc96; Biologend), and anti-CD127 BV421 (AO19D5; Biologend). Flow-Count Fluorospheres were purchased from Beckman Coulter, Miami, FL. Dead cells were excluded using the LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Life Technologies, New York, NY) as per the manufacturer's protocol.

Analysis of Immune Cell Populations Within the Blood and Skin

Blood and skin samples were transported on ice and processed within 2 hours of collection as previously described.¹² Briefly, to enable cell isolation from the skin, biopsies were cut into small pieces and then digested in preheated Roswell Park Memorial Institute 1640 Medium/2% FBS/3 mg/mL collagenase D/5 μ g/mL DNase 1 (Roche Applied Science, Victoria, Australia) for 90 minutes at 37°C. Samples were vortexed every 30 minutes to facilitate digestion. Cells were then passed through a 70- μ m cell strainer (BD Biosciences, Franklin Lakes, NJ) followed by a 40- μ m cell strainer (BD Biosciences). Lymphocytes were isolated from the blood by density centrifugation with Lymphoprep (Stemcell Technologies, Victoria, Australia) following the manufacturer's instructions. For analysis by flow cytometry, cells were incubated with 20- μ L Human Fc receptor binding inhibitor for 20 minutes on ice to prevent nonspecific antibody staining. Cells were then stained with monoclonal antibodies to identify TCR α/β ⁺ T cells, TCR α/β ⁺CD8⁺ CD8 T cells, TCR α/β ⁺CD4⁺ CD4 T cells, TCR α/β ⁺CD8⁺CD45RO⁺RA⁻ memory CD8 T cells, TCR α/β ⁺CD4⁺CD45RO⁺RA⁻ memory CD4 T cells, and

TCR α / β ⁺CD4⁺CD127^{lo}CD25^{hi} Treg cells. Immediately before fluorescence-activated cell sorting acquisition, flow-count fluorospheres were added to each sample to allow assessment of total cell counts. Analysis was performed using a Gallios Flow Cytometer (Beckman Coulter) and data analyzed using Kaluza software (version 1.2; Beckman Coulter). The gating strategy used (postfluorosphere gating) is outlined in **Figure S1, SDC**, (<http://links.lww.com/TXD/A44>). Investigators performing the flow cytometry were blinded to the study group from which the blood or skin sample originated. The results of total CD4 count were unavailable for 2 patients in the CKD group due to a technical problem with monoclonal antibody staining.

Statistical Analyses

Patient demographic and clinical characteristics were described using means, medians, SDs, interquartile ranges and proportions. Differences in demographic and clinical characteristics, categorized per immunosuppression exposure, were analyzed using chi-square, Fisher, analysis of variance, Kruskal-Wallis and Mann-Whitney tests where appropriate. Distribution of immune cell population numbers within the blood and skin were assessed using a Shapiro-Wilk normality test. Differences in immune cell populations between SE and non-SE skin within each group were analyzed using a paired t test for normally distributed data and a Wilcoxon signed-rank test for abnormally distributed data. Differences in immune cell populations between the SRL-treated KTRs, CNI-treated KTRs and CKD patients was analyzed using an analysis of variance test for normally distributed data and the Kruskal-Wallis test for abnormally distributed data. All statistical tests were 2-tailed, with a *P* value of less than 0.05 considered statistically significant. The correlations of T-cell population numbers between paired blood, SE and non-SE skin were analyzed using a 2-tailed Pearson correlation analysis after confirmation of normal distribution using a D'Agostino and Pearson omnibus normality test. Relationship strengths were described as follows: (*r*) > 0.5, strong; 0.3 to 0.5, moderate; -0.3 to -0.1 or 0.1 to 0.3, weak; -0.1 to 0.1, no relationship. Statistically significant correlations are indicated. Analyses were performed using STATA software version 13 (StataCorp., College Station, TX) and Prism version 6.0 (Graph-Pad, La Jolla, CA).

RESULTS

Patient Characteristics

Patient demographic and clinical characteristics are summarized in Table 1. KTRs receiving SRL or CNIs were more likely to have a history of skin cancer than CKD patients. There was no difference in history of nonskin malignancies between the groups. KTRs were more likely to have a history of glomerulonephritis compared with CKD patients whose causes of renal impairment were predominantly type 2 diabetes mellitus (*n* = 7) and polycystic kidney disease (*n* = 4). The groups were similar with respect to median age, sex, and serum creatinine levels. SRL- and CNI-treated groups were similar in terms of years of exposure to immunosuppression with a functioning transplant, history of rejection, and proportion of patients treated with azathioprine, mycophenolic acid, and prednisone.

TABLE 1.

Patient demographic and clinical characteristics

Characteristics	SRL (n = 15)	CNI (n = 15)	CKD (n = 15)	<i>P</i>
Age, y	61.3 ± 10	54.5 ± 11.3	59.3 ± 14.9	0.37
Male, n (%)	15 (100%)	13 (87%)	12 (80%)	0.34
Years of SRL or CNI therapy since current transplant (IQR)	5 (4-8)	5 (2-14)	—	0.56
Years of immunosuppression	11 (9-18)	13 (4-18)	—	0.80
Current azathioprine use	2 (13%)	4 (27%)	—	0.65
Current mycophenolic acid use	12 (80%)	10 (67%)	—	0.68
Current prednisone use	15 (100%)	15 (100%)	—	1.0
Current serum creatinine, μmol/L	113 (90-143)	129 (110-202)	156 (124-243)	0.10
Previous NMSC	14 (93%)	11 (73%)	3 (20%)	<0.01
Previous nonskin malignancy	1 (7%)	2 (13%)	1 (7%)	1.00
Glomerulonephritis as cause of primary kidney disease, n (%)	6 (40%)	7 (47%)	0 (0%)	0.01
Previous rejection	2 (13%)	5 (33%)	—	0.39

Results presented as mean ± SD, number (%) or median (IQR).

IQR, interquartile range; NMSC, nonmelanoma skin cancer.

SRL-Treated KTRs Display an Increased Abundance of Several T-Cell Subsets in SE Versus Non-SE Skin

Absolute numbers of CD8 T cells, memory CD8 T cells, CD4 T cells, memory CD4 T cells, and Treg cells were compared in paired samples of SE versus non-SE skin of CKD patients, SRL-treated KTRs and CNI-treated KTRs (Figure 1). The absolute number of these cell populations did not vary significantly per SE in CKD patients or patients treated with CNIs. In contrast, except for the total CD8 T cell count (Figure 1A), SRL treatment was associated with a statistically significant increase in the absolute number of memory CD8 T cells (Figure 1B), CD4 T cells (Figure 1C), memory CD4 T cells (Figure 1D), and Treg cells (Figure 1E) in SE skin as compared with patient-matched non-SE skin. These findings strongly suggest that systemic SRL treatment, but not CNI treatment, increases the absolute number of numerous T-cell subsets within SE skin as compared with non-SE skin.

Immunosuppressive Drug Usage Increases the Absolute Number of Memory T Cells and Treg Cells in the Skin But Not the Blood

Within each sample site (blood, non-SE and SE skin) absolute numbers of CD8 T cells, memory CD8 T cells, CD4 T cells, memory CD4 T cells and Treg cells were compared between CKD patients (the control group; no immunosuppressive drugs) and KTRs who received SRL or CNI treatment (Figure 2). Absolute CD8 T-cell numbers did not appear to differ significantly between patients on immunosuppressive drugs and the control group within any of the sample sites (Figure 2A). A significantly increased number of memory CD8 T cells were present in the SE skin of both SRL-treated and CNI-treated KTRs compared to controls (Figure 2B). Total CD4 T-cell numbers did not appear to differ between patients on immunosuppressant drugs and the control group within any of the sample sites (Figure 2C). A significantly increased number of memory CD4 T cells were present in

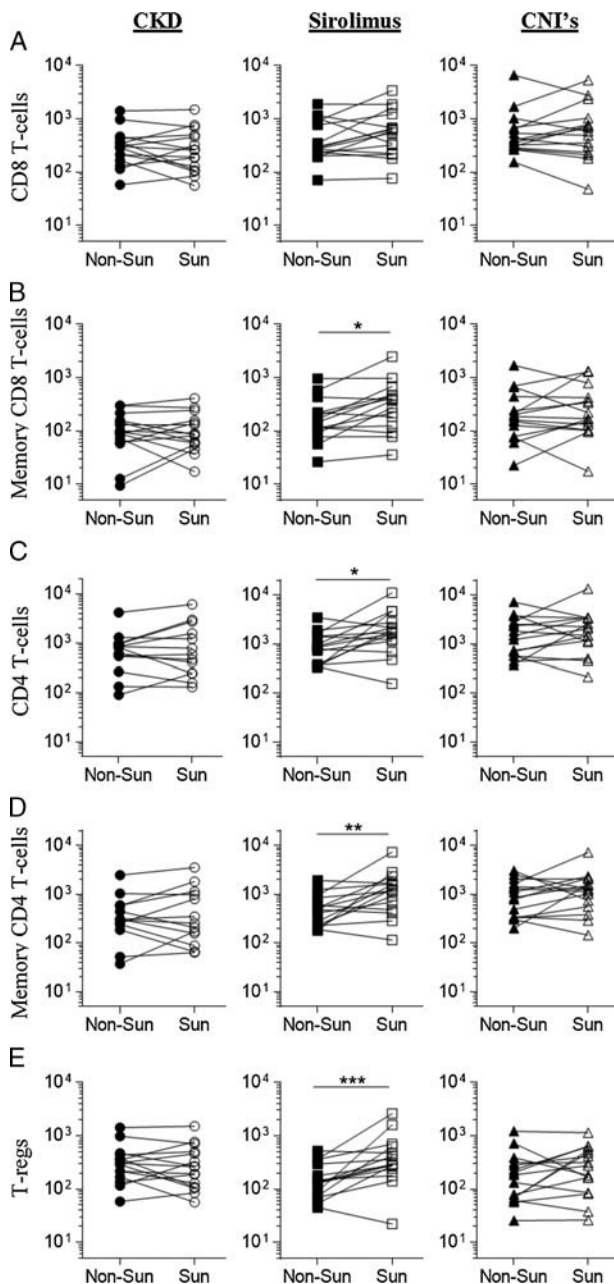


FIGURE 1. KTRs on SRL but not CNIs or patients with CKD display increases in the absolute number of T cell subsets in SE skin vs. non-SE skin. The absolute numbers of CD8⁺ T cells (A), memory CD8⁺ T cells (B), CD4⁺ T cells (C), memory CD4⁺ T cells (D), and Treg cells (E) were quantitated in patient-matched non-SE (nonsun; normal skin) and SE (sun; photodamaged) skin biopsies by flow cytometry as described in Materials and Methods. * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$. CKD (no immunosuppressive drugs), $n = 15$; SRL-treated KTRs, $n = 15$; CNI-treated KTRs, $n = 15$.

the non-SE skin of CNI-treated KTRs and a significantly increased number of memory CD4 T cells were present in the SE skin of both SRL- and CNI-treated KTRs compared with controls (Figure 2D). A significantly increased number of Treg cells were present in the SE skin of SRL-treated KTRs compared to controls (Figure 2E). Interestingly, there was no significant difference in the absolute number of CD8 T cells, memory CD8 T cells, CD4 T cells, memory CD4 T cells, or Treg cells in the blood of patients on immunosuppressant

drugs compared to the control group (Figures 2A–E). Together, the data suggest that immunosuppressant drug usage can significantly alter the absolute number of some T-cell populations in the skin but not the blood.

Prevalence of CD4⁺ T Cell Subsets in the Blood Does Not Accurately Predict Prevalence of CD4⁺ T-Cell Subsets in the Skin

Across the 3 patient groups the percentage of CD8 T cells (relative to total T cells), the percentage of memory CD8 T cells (relative to total CD8 T cells), the percentage of CD4 T cells (relative to total T cells), the percentage of memory CD4 T cells (relative to total CD4 cells), and the percentage of Treg cells (relative to total CD4 cells) was compared in blood and SE skin, and in blood and non-SE skin, in samples matched for the same patient (Figure 3). The percentage of

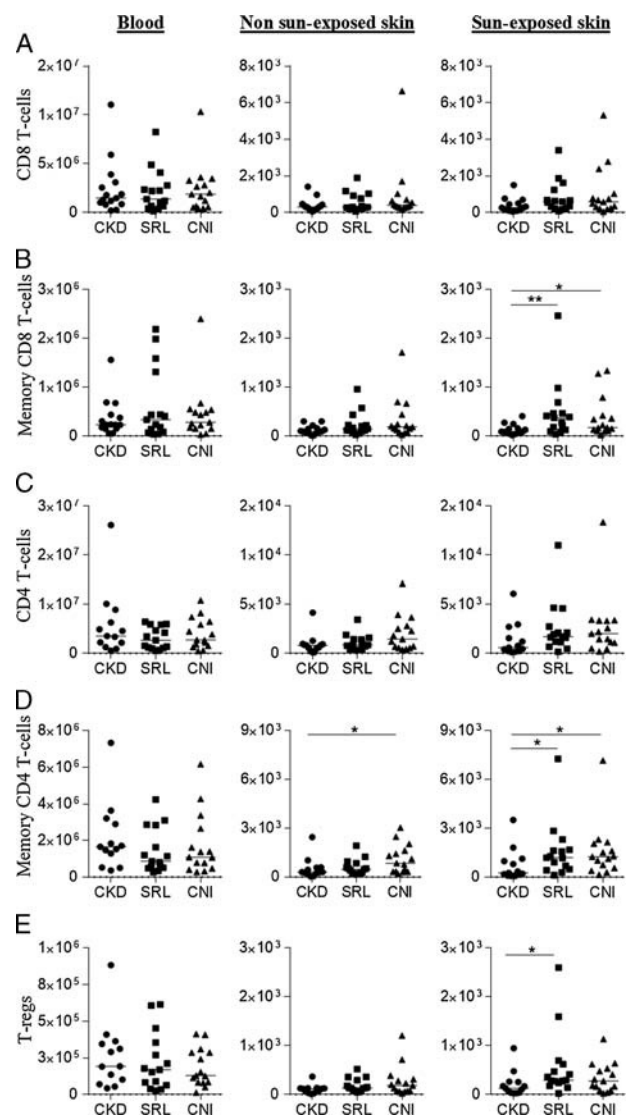


FIGURE 2. SRL and CNI treatment increases the absolute number of CD8⁺ and CD4⁺ memory T cells in the skin but not the blood. The absolute numbers of CD8⁺ T cells (A), memory CD8⁺ T cells (B), CD4⁺ T cells (C), memory CD4⁺ T cells (D), and Treg cells (E) were quantitated in patient-matched blood and non-SE and SE skin biopsies by flow cytometry as described in Materials and Methods. * $P < 0.05$ or ** $P < 0.01$. CKD (no immunosuppressive drugs), $n = 15$; SRL, $n = 15$; CNI, $n = 15$.

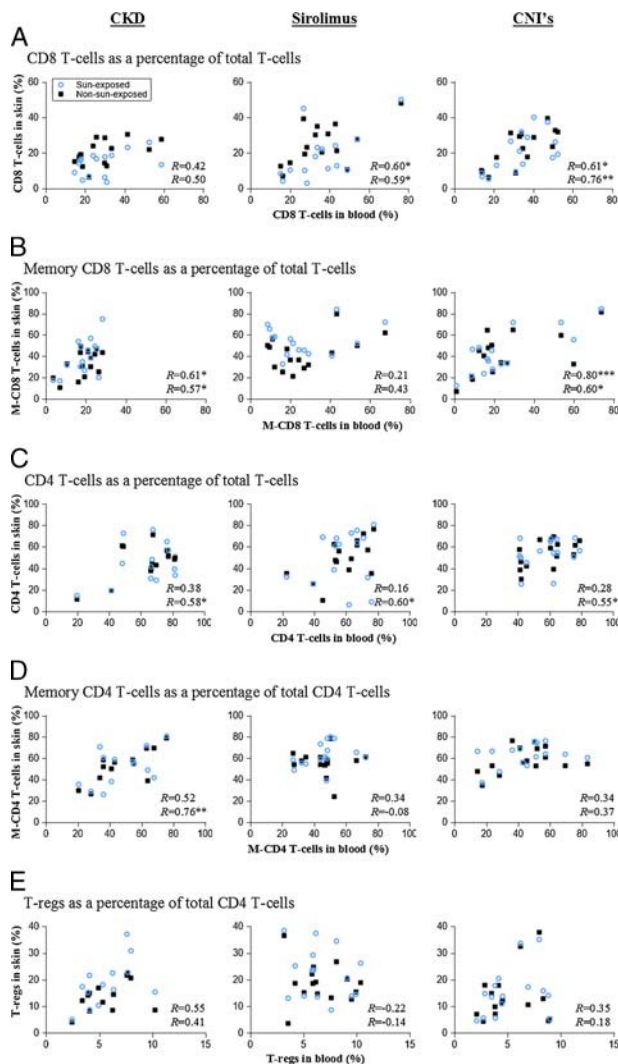


FIGURE 3. Percentage of T-cell subsets in the blood may not accurately predict percentage of T-cell subsets in the SE or non-SE skin of immunosuppressant drug-treated patients. Correlation analysis of T-cell abundance between the blood and skin. A, CD8⁺ T cells as a percentage of total T cells. B, memory CD8⁺ T cells as a percentage of total CD8⁺ T cells. C, CD4⁺ T cells as a percentage of total T cells. D, memory CD4⁺ T cells as a percentage of total CD4⁺ T cells. E, Treg cells as a percentage of total CD4⁺ T cells. Open blue circles (and upper correlation coefficient “r” values): Correlation of indicated T cell abundance between blood and SE skin. Closed black squares (and lower correlation coefficient “r” values): Correlation of T cell abundance between blood and non-SE skin. * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$. CKD (no immunosuppressive drugs), $n = 15$; SRL, $n = 15$; CNI, $n = 15$.

CD8⁺ T cells in both SRL and CNI-treated patients correlated strongly between the blood and SE (Figure 3A; SRL $r = 0.60$; CNI $r = 0.61$) and non-SE skin (Figure 3A; SRL $r = 0.59$; CNI $r = 0.76$). The percentage of memory CD8 T cells showed a similar strong correlation in CKD and CNI-treated patients across blood and SE (Figure 3B; CKD $r = 0.61$; CNI $r = 0.80$) and non-SE skin (Figure 3B; CKD $r = 0.57$; CNI, $r = 0.60$). In SRL-treated patients, however, a moderate correlation in memory CD8⁺ T-cell numbers was found between the blood and non-SE skin (Figure 3B; $r = 0.43$), whereas the correlation was weak between blood and SE skin (Figure 3B; $r = 0.21$). Irrespective of the presence or absence of

immunosuppression, the percentage of CD4 T cells in the blood did not show a significant correlation with that in SE skin (Figure 3C; CKD, $r = 0.38$; SRL, $r = 0.16$; CNI, $r = 0.28$), although a strong correlation was seen between the percentage of CD4 T cells in the blood and non-SE skin (Figure 3C; CKD, $r = 0.58$; SRL, $r = 0.60$; CNI, $r = 0.55$). There was a lack of correlation between the percentage of memory CD4⁺ T cells in the blood and SE skin across all groups (Figure 3D). However, in contrast to patients not receiving immunosuppression (Figure 3D; CKD $r = 0.76$), both SRL and CNI-treated KTRs failed to show a significant correlation between the percentage of memory CD4⁺ T cells in the blood and non-SE skin (Figure 3D; SRL $r = -0.08$; CNI $r = 0.37$). Interestingly, irrespective of the presence or absence of immunosuppression, no statistically significant correlations were found between the percentage of Treg cells in the blood and SE (Figure 3E; CKD $r = 0.55$; SRL $r = -0.22$; CNI $r = 0.35$) or non-SE skin (Figure 3E; CKD $r = 0.41$; SRL $r = -0.14$; CNI $r = 0.18$). Altogether, these data suggest that whether measurement of T cell abundance in the blood accurately reflect that in the skin may be associated with the T cell subset under examination, whether the skin has been SE, and the type of immunosuppressive medication the patients have received.

In SRL-Treated KTRs the Prevalence of CD4⁺ T Cells Correlates Poorly Between SE and Non-SE Skin

To determine how chronic SE might impact the abundance of T-cell subsets in the skin, we performed an inpatient correlation analysis between SE and non-SE skin sites derived from the same patients. As shown in Figure 4, we observed a strong correlation in the percentage of CD8 T cells (relative to total T cells) (Figure 4A; CKD, $r = 0.76$; SRL, $r = 0.83$; CNI, $r = 0.81$) and the percentage of memory CD8⁺ T cells (relative to total CD8 T cells) (Figure 4B; CKD, $r = 0.58$; SRL, $r = 0.74$; CNI, $r = 0.77$) between skin sites irrespective of the presence or absence of immunosuppression. We also observed a strong correlation in the percentage of CD4⁺ T cells (relative to total T cells; Figure 4C; CKD, $r = 0.86$; CNI, $r = 0.59$) and the percentage of memory CD4 T cells (relative to total CD4⁺ T cells; Figure 4E; CKD, $r = 0.60$; CNI, $r = 0.82$) between SE and non-SE sites in nonimmunosuppressed patients and patients on CNIs. In contrast, SRL-treated patients did not show a statistically significant correlation in the percentage of CD4 T cells (relative to total T cells; Figure 4C; SRL, $r = 0.42$) or memory CD4 T cells (relative to total CD4 T cells; Figure 4D; SRL, $r = 0.07$) between SE and non-SE sites. Interestingly however, the percentage of Treg cells (relative to total CD4 T cells; Figure 4E; CKD, $r = 0.72$; SRL, $r = 0.77$; CNI, $r = 0.92$) correlated strongly between these 2 skin sites regardless of the presence or absence of immunosuppression. The data suggest that SRL, but not CNIs, may disrupt the balance of CD4⁺ T cells, particularly memory CD4⁺ T cells, in the skin after chronic sun exposure.

DISCUSSION

In this study, correlation between blood and skin immunophenotype was dependent on the T cell subset under investigation, whether or not the skin was SE, and the type of immunosuppressive treatment received. An improved understanding of how the immune-phenotype in peripheral blood correlates with that in the skin might allow clinicians to use

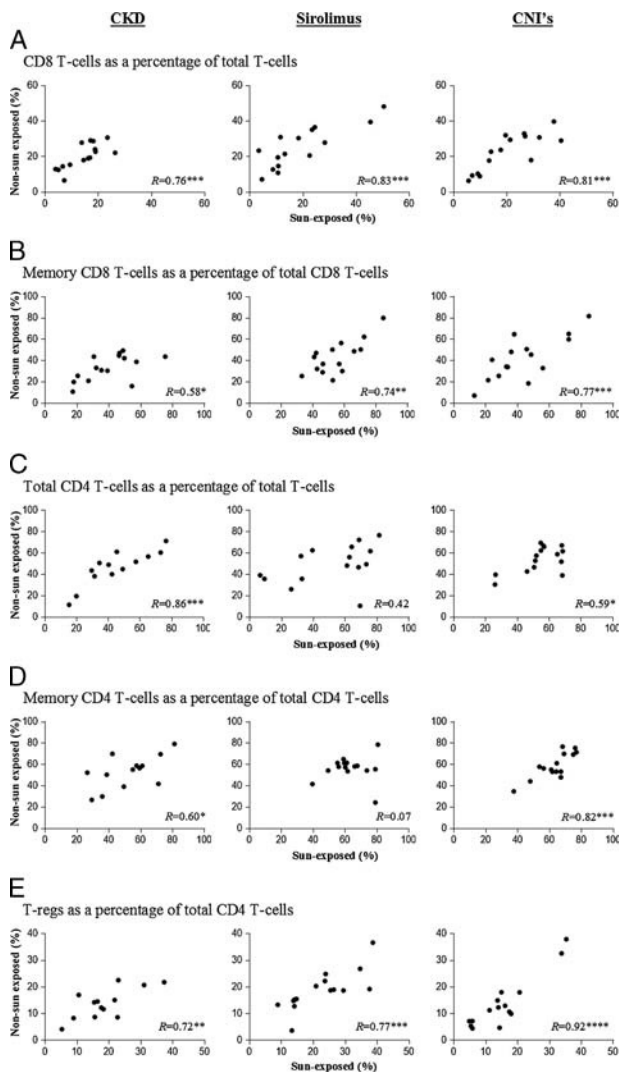


FIGURE 4. In SRL-treated KTRs, the percentage of CD4⁺ T cells (relative to total T cells) and the percentage of memory CD4⁺ T cells (relative to total CD4⁺ T cells) correlates poorly between SE and non-SE skin. A, CD8⁺ T cells as a percentage of total T cells, (B) memory CD8⁺ T cells as a percentage of total CD8⁺ T cells, (C) CD4⁺ T cells as a percentage of total T cells, (D) memory CD4⁺ T cells as a percentage of total CD4⁺ T cells, and (E) Treg cells as a percentage of total CD4⁺ T cells. “r” values = correlation coefficient. **P* < 0.05; ***P* < 0.01; or ****P* < 0.001. CKD (no immunosuppressive drugs), n = 15; SRL, n = 15; CNIs, n = 15.

immune-phenotyping, from easily obtained blood samples, to adjust immunosuppressive therapy and tailor immunosuppression for individual patients. The last 30 years has seen an increasing potency of immunosuppressive therapy and a corresponding reduction in acute rejection episodes. However, this has occurred at the expense of an increase in deaths secondary to infection and malignancy. The incidence of skin cancer is greatly increased after solid organ transplantation and so manipulation of immunosuppression based on individualized peripheral blood immune-phenotyping could be of great benefit. In this study, the percentage of memory CD8 T cells (relative to total CD8 T cells) showed good correlation between skin and blood in CKD and CNIs groups. However, this study also demonstrated that the percentage of memory CD8⁺ T cells showed poor correlation between

blood and skin in SRL-treated patients, and the percentage of Treg cells (relative to total CD4 T cells) showed poor correlation between blood and skin regardless of immunosuppressive exposure. Therefore, peripheral blood immune phenotyping is not a reliable predictor of Treg cell numbers in the skin, irrespective of type of immunosuppressive exposure, or of memory CD8⁺ T cell or memory CD4⁺ T cell numbers in those KTRs treated with SRL.

Our study also found that the type of immunosuppressive drug treatment and whether or not the skin was SE affected immune cell population frequencies. Memory CD8⁺ T cells and memory CD4⁺ T cells were more frequent in the skin of SRL- and CNIs-treated patients compared with CKD patients. Intra-patient analysis revealed that memory CD8⁺ T cells, CD4⁺ T cells, memory CD4⁺ T cells, and Treg cells were all increased in the SE compared with non-SE skin of SRL patients, but not in CKD or CNIs patients. The positive effects of SRL on memory CD8 T cell and Treg cell development have been documented previously⁴; however, the contribution of SE in this process is less well defined. Possible effects of SE are supported by a study of 20 Norwegian psoriasis patients who after (acute) sun exposure in Spain had a reduction in CD8⁺ T cells in epidermis and dermis and a relative increase in FOXP3⁺ T cells.¹³ Furthermore, a murine study showed that UVB exposure before contact sensitization, inhibited the expansion of effector CD4⁺ and CD8⁺ T cells in skin-draining lymph nodes and reduced the number of CD4⁺ and IFN- γ ⁺ CD8⁺ T cells in the challenged ear skin.¹⁴ In the absence of UVB, after hapten exposure, the skin was infiltrated by effector memory CD8⁺ T cells at the site of challenge. However, if mice were previously exposed to UVB this cell population was absent (suggesting an impaired development of peripheral memory T cells). This immunosuppressive effect of UV light on memory CD8 T cells is supported by an earlier study which showed that suberythemal solar simulated UV light reduced the response to purified protein derivative in mantoux positive participants.¹⁵

Manipulation of immune phenotype with UV radiation has been extensively studied as a treatment for a broad range of diseases. Extracorporeal photopheresis (ECP) is a leukapheresis technique using UVA irradiation and 8-methoxypsoralen. It has been used for the treatment or prevention of renal¹⁶ and cardiac transplant rejection,¹⁷ graft versus host disease¹⁸ and cutaneous T cell lymphomas.¹⁹ A study of 60 cardiac transplant recipients randomized to standard immunosuppressive therapy alone or in conjunction with photopheresis reported a significant reduction in acute rejection in patients receiving photopheresis.¹⁷ Although the exact immunosuppressive mechanism of ECP is uncertain, it is most likely due not only to apoptosis of immune cells²⁰ but also the immunosuppressive effects of Treg cells.²¹⁻²⁴

Supporting our findings in the SE skin of SRL patients, a study of graft-versus-host-disease patients showed the frequency of Treg cell in peripheral blood increases after each cycle of ECP.²⁵ Whether SRL treatment enhances the development of CD4⁺ Treg cells in skin along with UV exposure or whether CNIs treatment impairs development remains an important unanswered question.

In this study, we found no impact of immunosuppression on the abundance of T cell subpopulations in the blood. Recently, Gallon et al⁴ examined the frequency of total T cells, naïve and memory CD8⁺ and CD4⁺ T cells,

and CD4⁺CD25⁺FOXP3⁺ Treg cells in the blood of KTRs converted from tacrolimus to SRL. The authors reported no effect of drug conversion on the abundance of any T cell subpopulation in the blood except for Treg cells, which were increased in the SRL-converted group. It should be noted, however, that Gallon et al identified Treg cells in their study by positive staining for FOXP3. Although we have also used this approach in the past,²⁶ we opted to define Treg cells as CD4⁺CD127^{lo}CD25^{hi} in the current study based on reports that in humans, FOXP3 can also be marker of T cell activation.²⁷⁻²⁹ Intriguingly, previous studies defined an association between CD4⁺FOXP3⁺CD127^{lo}CD25^{hi} Treg cells and SCC incidence in KTRs,^{10,30} although this association was lost when Treg cells were defined as CD4⁺FOXP3⁺CD127^{lo}CD25^{hi}Helios⁺ (approximately 80% of the CD4⁺FOXP3⁺CD127^{lo}CD25^{hi} population).³⁰ At least 1 further study has reported no significant effect of SRL as compared with CNI on Treg cell abundance in patient blood. In summary, given study-to-study differences in Treg cell-defining markers, it does not seem surprising that our findings regarding Treg cell abundance in the blood do not align with that of Gallon et al.

This study has several limitations. Ideally, KTRs would have been randomized to receive either SRL or a CNI to prevent indication bias. In this study, KTRs receiving SRL were more likely to have a history of NMSC and therefore differences in immune cell populations between treatment groups may be due to previous skin cancer; or patients with an immune phenotype being more likely to develop cancer and therefore receive SRL treatment. The relatively small group sizes led to different proportions of male and female patients, which may also have influenced the findings because sex is known to have effects on susceptibility to the immune modulating effects of UV radiation. Although there was no difference between the proportions of patients also receiving azathioprine, mycophenolic acid or prednisone in SRL- and CNI-treated KTRs; these combinations of immunosuppressants may well have also had effects on immune cell populations. A further limitation is the lack of functional data because of the low numbers of T cells available in patient skin biopsies. It seems probable that even if the abundance of T cells in SRL or CNI patients are similar that they function very differently as a consequence of different mechanisms of suppression, and therefore, as pointed out previously, the effectiveness of the immune system in KTRs should not be gauged by cell numbers alone.¹²

CONCLUSIONS

This is the first study to examine the validity of performing flow cytometry in peripheral blood to predict tissue specific immune phenotype. Correlation between skin and blood varied for different immune cell populations but was poor for CD4 Treg cell population numbers. Chronic sun exposure influenced immune phenotype in skin with an increase in multiple T cell subsets in SRL-treated patients, an increase in memory CD8 and memory CD4 T cells in SRL-treated and CNI patients; and an increase in Treg cells in SRL but not CNI patients. Immune cell populations in skin are therefore likely to be differentially influenced by both sun exposure and patient immunosuppressive treatment.

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