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Characterization of T cell immunophenotypes in intestinal transplantation: A pilot study

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Abstract

Immunophenotyping of peripheral blood mononuclear cells has been shown to be a useful, non-invasive method of predicting acute cellular rejection (ACR) following intestinal transplantation (ITx). Our objectives were to characterize differences in the T cell immunophenotype of ITx recipients in peripheral blood samples (1) collected late versus early after ITx and (1) associated with episodes of ACR and infectious enteritis. An IRB-approved, cross-sectional study of ITx recipients was performed. Peripheral blood samples were collected during normal visits and episodes of allograft dysfunction. A total of 38 patients were included in the analysis: 31 ITx recipients (87% liver-inclusive allografts) and 7 intestinal failure control patients. Of the ITx patients, 26 patients were pediatric patients (< 21 years). A total of 70 samples were analyzed from ITx recipients, including 51 during normal visits and 19 during episodes of allograft dysfunction (median of 2 samples per patient; range of 1–6 samples per patient). In the late (n = 32) versus early post-ITx (n = 19) normal samples, there was a significantly higher percentage of central memory CD4 T cells (p = .001). In the ACR (n = 5) versus infectious enteritis (n = 14) samples, there was a higher percentage of CD8 T cells expressing HLA-DR (p = .002), CD57 (p < .001), and KLRG1 (p < .001) and a higher percentage of CD4 T cells expressing CD57 (p = .03). Additional studies are needed with larger cohorts to validate these changes in the T cell immunophenotype. Further elucidating T cell immunophenotypes in ITx will lead to a better understanding of immune mechanisms of allograft dysfunction, identification of potential biomarkers in ITx, and optimized selection of immunosuppressive therapies.

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Declarations of interest

None.

Keywords

Intestinal transplantation; Immune monitoring; Immunophenotyping; T cell; Infection; Rejection

1. Introduction

Intestinal transplantation (ITx) is a lifesaving procedure for patients with irreversible intestinal failure who have complications of parenteral nutrition, an inability to adapt to the quality-of-life limitations posed by intestinal failure, and/or a high risk of death if the native intestine is not removed [1]. Modest improvements have been made in early graft survival rates over the past decade, but rates of allograft loss beyond one year have not improved [2]. A significant barrier to successful ITx is acute cellular rejection (ACR). ACR continues to affect almost 40% of ITx recipients in the first year after transplantation [3] with potentially devastating consequences. ACR also remains an important cause of late allograft loss [4].

In the setting of intestinal allograft dysfunction, a major challenge is the differentiation of ACR from infectious enteritis. Infectious enteritis is a common cause of allograft dysfunction, occurring in up to three quarters of pediatric ITx recipients [5]. Although the gold standard for diagnosis of ACR is endoscopy with biopsy to identify key histopathologic findings [6], there are inherent disadvantages to endoscopy. Histopathology obtained from endoscopy is subject to sampling and interpretation error [7], with newer grading scores being proposed [8]. In addition, endoscopy is associated with higher risks of complications in pediatric ITx recipients [9] as well as a significant burden of cost [10]. Thus, there remains a clear need for a non-invasive means to discriminate between ACR and infectious enteritis.

Although multiple prior attempts to develop non-invasive biomarkers of immune monitoring in ITx have been suboptimal [11–14], immunophenotyping of T cells has been shown to be a useful, noninvasive method of predicting ACR in pediatric ITx [15,16]. Peripheral blood mononuclear cells (PBMCs), including T cells, offer a unique window through which the recipient's immune response to transplanted tissue can be monitored non-invasively. These cells are present in the peripheral blood after allo-endothelial-cell contact in the allograft due to recirculation. Immunophenotyping of specific T cell subsets have also been associated with ACR in pediatric liver transplantation [17,18] and adult renal transplantation [19].

We performed a cross-sectional, single center, pilot study to monitor peripheral blood T cell immunophenotypes at various time intervals following ITx and across clinical states (ACR, infectious enteritis, and normal baseline stability).

2. Objectives

The objectives of this study were to [1] characterize differences in the peripheral blood T cell immunophenotype of ITx recipients in normal samples collected late versus early after ITx and [2] characterize differences in the peripheral blood T cell immunophenotype associated with episodes of ACR and infectious enteritis.

3. Materials and methods

3.1. Study design

This was a prospective, cross-sectional study of ITx recipients transplanted from 2000 to 2016. Samples were collected from 2011 to 2017. The study was approved by the University of California, Los Angeles (UCLA) institutional review board, IRB #12–001231.

3.2. Study population and sample collection

Informed consent was obtained from patients and/or their parents at the time of enrollment. All patients followed by the UCLA Intestinal Transplant Program who received an ITx, with or without a liver allograft, were eligible for the study. Patients were excluded from the study if they had previously received alemtuzumab due to its potent leukocyte depletion effect. Samples were excluded if the patient had received anti-thymocyte globulin (ATG) within the past six months and/or had a diagnosis of or treatment for post-transplant lymphoproliferative disorder (PTLD) within the past three months. Samples were also excluded if they met criteria for both ACR and infectious enteritis concomitantly. Importantly, enrollment took place at any time point after ITx and sample collection occurred at various time points thereafter. As a control group, patients with intestinal failure followed at UCLA for ITx evaluation and/or for management of home parenteral nutrition were also recruited into the study.

Peripheral blood samples were collected post-ITx from each patient at clinical visits or during hospitalizations under two conditions throughout the study period as follows:

Samples were collected from ITx recipients when they were asymptomatic with stable intestinal allograft function on baseline immunosuppression, which were designated as normal samples. Subsequent sample(s) were collected at least six months apart in a subset of patients. To assess for differences in the T cell immunophenotype in relation to *time after ITx*, these samples were further grouped based on the date of collection in relation to time of transplantation: early post-ITx (collected < 5 years after ITx) and late post-ITx (collected > 5 years after ITx).

Allograft dysfunction samples were collected when ITx recipients presented to medical attention with abnormal intestinal allograft function. This was defined as the presence of high fecal outputs above baseline, change in consistency of fecal output, nausea/vomiting, ileus/ obstruction, and/or gastrointestinal bleeding. Stool studies with or without endoscopy were performed at the discretion of the clinical team. Peripheral blood samples were obtained prior to treatment for ACR or infectious enteritis, if applicable. To assess changes in the T cell immunophenotype *across varying clinical states*, these samples were further grouped based on the etiology of allograft dysfunction as described below.

ACR was defined as biopsy-proven rejection classified according to the standardized grading scheme as defined at the VIII International Small Bowel Transplant Symposium [6] or indeterminate histopathology but high clinical suspicion such that the patient was treated for ACR. Infectious enteritis was defined based on positive stool studies and/or endoscopy findings. Viral enteritis was defined as fecal Nor-ovirus PCR, Adenovirus antigen, Rotavirus

antigen screen, and/or viral culture positive. Evaluation for CMV enteritis was performed either via viral culture or endoscopic biopsies, including evaluation for viral inclusions and CMV immunostaining. Bacterial enteritis was defined as *Clostridium difficile* PCR and/or bacterial pathogenic PCR positive.

To assess for the effect of transplantation on T cell subsets, control samples were also collected at one time point from intestinal failure patients without intestinal allografts who were at their clinical baseline without signs of infection. The reason for using intestinal failure patients rather than healthy controls was to account for any differences in immune memory that may have been affected by past abdominal surgeries and/or infectious complications (including episodes of sepsis, cathether related bloodstream infections, and nosocomial infections).

3.3. T cell immunophenotyping

Peripheral blood mononuclear cells were separated by Ficoll. Cells were frozen in Human Serum 10% DMSO until flow cytometry immunophenotyping was carried out. Flow cytometry was performed by the UCLA Immune Assessment Core. 0.5×10^6 cells per cocktail were stained for 20 min at 4 °C with the 1:1000 LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Life Technologies). Cells were then washed once with FACS buffer (PBS supplemented with 2% FBS) and stained with fluorochrome-conjugated antibodies (BioLegend, BD Biosciences, eBioscience) for 20 min at 4 °C. Finally, the cells were washed and resuspended in 200 μl FACS buffer. If possible, > 100,000 lymphocyte events per sample were acquired using DIVA 8.0 software on LSRFortessa Cell Analyzer (BD Biosciences). Data analysis was performed using FlowJo VX.0.7r2 (Tree Star) by gating on live cells based on forward versus side scatter profiles, then gating on singlets using forward scatter area versus height, followed by dead cell exclusion using Live/Dead exclusion stain, and then T cell subset-specific gating. T cells were gated as CD3+CD4+ or CD3+CD8+ cells, then subsequently differentiated with the following markers of interest: naïve (CCR7+CD45RA+), effector memory (CCR7-CD45RA−), central memory (CCR7+CD45RA−), activated (HLA-DR1+), antigen-experienced/senescent (KLRG1+), chronically activated/senescent (CD57+), activated suppressive/exhausted (PD1+). See Table 1 for antibody-fluorochrome panel used and Fig. 1 for gating strategy.

3.4. Data collection

Demographic (age, sex, race), clinical (primary etiology of intestinal failure, liver-inclusive or non-inclusive allograft, age at the time of ITx, age at the time of sample collection, immunosuppression at the time of sample collection, induction therapy), laboratory (complete blood cell count, comprehensive metabolic panel, tacrolimus level, EBV DNA PCR and CMV DNA PCR), and pathology data (if available) was collected on each patient at the time of sample collection.

3.5. Statistical analysis

For objective 1, differences between T cell subsets were analyzed in normal samples of the late post-ITx versus early post-ITx groups. Age at the time of sample collection was added to the model to account for changes in naïve and memory cells that may be attributed to age.

To assess the effect of ITx on changes over time, differences between T cell subsets were analyzed in normal samples from ITx recipients and samples from intestinal failure patients. To assess the effect of immunosuppression, the presence or absence of each immunosuppressive agent on T cell subsets was individually analyzed. For objective 2, differences between T cell subsets were analyzed in ACR versus infectious enteritis samples, ACR versus normal samples, and infectious enteritis versus normal samples.

Descriptive statistics were used to summarize the collected data: continuous variables were summarized using their medians and interquartile ranges; categorical variables were summarized using counts of observations that fall into each group and their percentages. Univariate analysis was performed by using a Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables where a p-value < .05 was considered statistically significant (Table 2). To compare the T cell immunophenotyping values between different groups, we used generalized linear mixed-effect models with random intercept for each patient. This approach takes into account that each patient can have multiple samples. Since age may affect immunophenotype, it was included in the model as a controlled variable.

For each immunophenotyping marker we report the mean difference, 95% confidence intervals, p-values (Tables 3, 4, 5). To control for false discovery rate (FDR), we report FDR-adjusted p-values in our tables in addition to unadjusted p-values. Given that this was a hypothesis-driven, exploratory study, the unadjusted p-value is presented throughout the text. Statistical significance was defined as an unadjusted p-value < .05. We used R software (version 3.4.4) to perform the analysis.

3.6. Immunosuppression management

Our protocol for immunosuppression has been described elsewhere [20]. In brief, since 1999, induction therapy consists of an IL2 receptor antagonist (IL2RA) for 6–8 weeks post-transplant in normal-risk recipients and rabbit ATG or alemtuzumab in high-risk recipients (liverfree allografts, presensitized, and re-transplant patients). Maintenance immunosuppression is tacrolimus (Prograf; Astellas, Deerfield, IL) – based and also includes mycophenolate mofetil (Cellcept; Genentech, San Francisco, CA) or sirolimus (Rapamune; Pfizer, Philadelphia, PA). For induction and maintenance, all recipients are given corticosteroids which are typically weaned off in 1–3 years.

4. Results

4.1. Study population

The study population was comprised of 38 patients: 31 ITx recipients and 7 intestinal failure control patients. The ITx cohort included 26 children and 5 adults. Five of the patients were re-ITx recipients; 87% of ITx recipients had a liver-inclusive allograft. The most common underlying etiology of intestinal failure was surgical short bowel syndrome in the ITx recipients, with the most common diagnosis of gastroschisis (n = 8). Comparatively, the most common underlying etiology of intestinal failure was motility disorder in the intestinal failure patients, with the most common diagnosis of chronic intestinal pseudo-obstruction (n

= 4). The clinical characteristics of the ITx and intestinal failure cohorts are summarized in Table 2.

4.2. Sample characteristics

A total of 70 peripheral blood samples from the 31 ITx recipients were analyzed, with a median of 2 samples per patient (range of 1–6 samples per patient). The median time since ITx at which samples were collected was 85.2 months (IQR 41.4–132.3). At least one normal sample was collected from 30 ITx recipients. A second normal sample was collected in 11 patients and > 2 subsequent samples were collected in 5 patients, for a total of 51 normal samples (range of 1–4 normal samples per patient). Only 5 samples were collected during the first year post-transplant, with 14 samples collected between years 1–5, 15 samples between years 5–10, and 14 samples between years 10–15, and 3 samples collected after 15 years. Of the normal samples, 19 were categorized into the early post-ITx group (< 5 years after ITx) and 32 were categorized into the late post-ITx group (> 5 years after ITx). One sample from each of the intestinal failure patients was collected for a total of 7 control samples.

A total of 19 samples were collected during episodes of allograft dysfunction. Five samples met criteria for ACR and 14 samples met criteria for infectious enteritis. Of the infectious enteritis samples, four were attributed to *Clostridium difficile* infection while the remainder were attributed to viral enteritis: Norovirus [4], Adenovirus [2], Rhinovirus/Enterovirus [2], Other [2].

4.3. Immunophenotyping: differences in normal samples collected late versus early post-ITx

Table 3 demonstrates differences between the percentage of naïve and memory T cells between the late versus early post-ITx normal samples. As shown in Fig. 2, among the normal samples from ITx recipients, there was an increased percentage of central memory CD4 T cells in the late versus early post-ITx groups: difference 11.4, 95% CI (4.7, 18.2), $p = .001$.

The median time since ITx at which the sample was collected was 29.1 months (IQR 13.4–41.7) in the early post-ITx group and months (IQR 93.6–146.6) in the late post-ITx group. The median age at the time of sample collection was 10.9 years (IQR 7.2–20.1) in the early post-ITx group and 13.6 years (IQR 10.2–16.0) in the late post-ITx group. As shown in Table 4, the percentage of central memory CD4 T cells was not affected by the presence or absence of individual immunosuppressive therapies (corticosteroid, mycophenolate mofetil, sirolimus).

In the normal ITx samples compared to the intestinal failure control samples, there was a significantly lower percentage of naïve CD4 T cells: difference −15.6, 95% CI (−29.9, −1.3), $p = .03$. There was also a significantly higher percentage of effector memory CD4 T cells: difference 10.9, 95% CI (3.9, 18.0), $p = .002$ (Table 5).

4.4. Immunophenotyping: across clinical states

As shown in Table 6, the percentage of CD8 T cells expressing HLA-DR, CD57, KLRG1, and PD1 was significantly higher in the ACR versus normal samples. The percentage of CD8 T cells expressing HLA-DR, CD57, and KLRG1 was also significantly higher in the ACR compared to infectious enteritis samples. Similarly, the percentage of CD4 T cells expressing HLA-DR and CD57 was significantly higher in the ACR versus normal samples. The percentage of CD4 T cells expressing CD57 was also significantly higher in the ACR samples compared to infectious enteritis samples. Also, fewer CD8 naïve cells were seen in ACR samples when compared with normal samples: difference −25.7. 95% CI (−44.5, −7.0), $p = .007$ (adjusted p-value = .05).

The median time since ITx at which the sample was collected was 85.2 months (IQR 30.0–131.9) in the ACR group and 88.5 months (IQR 79.5–104.9) in the infectious enteritis group. The median age at the time of sample collection was 12.0 years (IQR 10.2–12.1) in the ACR group and 8.9 years (IQR 7.9–11.9) in the infectious enteritis group.

5. Discussion

The study reported herein features immunophenotyping data from a relatively large cohort of ITx recipients at a single center. ITx is an uncommon procedure, reserved for a subset of intestinal failure patients who have developed complications of parenteral nutrition. The most recent data from OPTN/SRTR states that, as of 2016, there were almost 1200 ITx recipients living with a functioning allograft, with a total of 147 intestinal transplants performed in that year [3]. Thus, clinical research on ITx patients is limited due to the rarity of the procedure. Our study represents a much-needed analysis of immune markers of the intestinal allograft in a relatively large ITx population. We employed immunophenotyping to assess for differences in samples relative to time of transplantation. We observed an increase in central memory CD4 T cells in normal samples collected late compared to early after ITx. This study also assessed for differences between two of the most common etiologies of intestinal allograft dysfunction: ACR and infectious enteritis. In ACR compared to infectious enteritis samples, we observed a higher proportion of HLA-DR+, CD57+, and KLRG1+ CD8 T cells and a higher proportion of CD57+ CD4 T cells.

In transplant recipients, the immunophenotype is influenced by immune experience, or prior immune responses [21]. Memory T cells play an important role in allograft rejection and are an important barrier to tolerance [22]. Notably, memory T cells underlie “heterologous cross-reactivity”, or the concept that infection can lead to memory T cells that can cross-react with other similar but non-identical antigens, potentially leading to an increased risk of alloimmune responses [23]. In fact, specific therapy against memory T cells may help prevent allograft rejection [24]. Thus, our understanding of memory cells in ITx, and how they shift over time after transplantation, is critical in determining appropriate immunosuppressive regimens for each individual patient.

In our cohort, we observed an increase in central memory CD4 T cells in samples collected late after ITx, likely related to immune experience. Nearly three-quarters of pediatric ITx recipients experience at least one episode of ACR and almost 80% of patients experience an

episode of infectious enteritis [5]. Importantly, we did not see an effect of immunosuppression on central memory CD4 T cells associated with individual immunosuppressive therapies. More complex multivariable analysis on immunosuppression was unable to be performed due to small sample size. We did not see a difference in central memory CD4 T cells in comparing the intestinal failure and ITx groups. However, we did observe that ITx was associated with less naïve and more effector memory CD4 T cells which may be due to heightened immuno-reactivity following ITx. Overall, this suggests that our current immunosuppressive regimens may be inadequate in preventing the memory response after ITx.

ACR continues to be a significant barrier to successful ITx. At the same time, determining a balance between over-and under-immunosuppression is critical. Too much immunosuppression can lead to complications including infection. Currently, there are few non-invasive tools available to assess immune activity of the intestinal allograft. Proteomic and cytokine analysis of ostomy effluent has demonstrated the critical role of the innate immune system during episodes of ACR [25]. In addition, the Pleximmune™ test (Plexision Inc., Pittsburgh, PA, USA) is an established, FDA approved assay that employs immunophenotyping of allospecific CD154+ T-cytotoxic memory cells to identify pediatric ITx recipients who are at risk for rejection [26,27].

T cell exhaustion and senescence represent distinct states in the differentiation of T cells, particularly in the setting of chronic antigen stimulation. T cell senescence is defined as a state in which differentiated T cells lose their proliferative capacity while presumably retaining their capacity to secrete pro-inflammatory cytokines upon stimulation [28]. It is characterized by specific markers including CD57+ and KLRG1+. T cell exhaustion is defined as a differentiated state in which T cells lose their effector functions in the setting of persistent antigen exposure and/or inflammation [29]. It is characterized by upregulation of the inhibitory receptor PD1+. In the setting of persistent antigen stimulation, PD1+ is upregulated early during T cell activation, while the T cell still retains cytolytic and proliferative effector function [30]. PD1+ expression increases as the differentiation into T cell exhaustion progresses to loss of effector function. Thus, PD1+ may either represent an early T cell activation marker or a response to stimulation from persistent antigen exposure leading to an exhausted T cell phenotype.

Both CD57+ and PD1+ have been linked to rejection risk following renal transplantation. Espinosa et al. found that CD57+ CD4 T cells present prior to renal transplantation underlie belatacept-resistant allograft rejection [31]. In addition, they demonstrated that CD57+ CD4 T cells represent a non-senescent, cytolytic phenotype distinct from the senescence associated with CD57 expression on CD8 T cells [32], consistent with their role in the inflammatory process of rejection. A separate study in renal transplantation identified PD1 expression on T cells as an indicator of rejection risk, particularly in the pre-transplant period [33]. Both studies suggest that PD1 and CD57 expression may serve as potential biomarkers of T cell activation prior to transplantation, portending an immuno-reactive state that lends itself to ACR following transplantation.

In our study, we found an increase in markers of exhaustion and senescence during episodes of ACR when compared to normal samples collected during clinical stability. We also observed a persistent increase in HLA-DR+, CD57+, and KLRG1+ of CD8 T cells as well as CD57+ of CD4 T cells during episodes of ACR compared to episodes of infectious enteritis. The upregulation of HLA-DR, a generalized marker of T cell activation independent of the other aforementioned markers, suggests that CD57+, KLRG1+, and PD1+ represent a state of T cell activation rather than a state of exhaustion and senescence. The fact that some of the activation markers are higher in ACR versus infectious enteritis might also suggest that systemic effects are more marked in ACR when compared to infectious enteritis. It remains to be seen whether this means that infectious enteritis is a more localized inflammatory event. Interestingly, the absence of a difference in the expression of PD1 between ACR and infectious enteritis suggests that PD1+ is also upregulated in infectious enteritis, consistent with prior studies describing PD1+ T cells in the setting of chronic infection after transplantation [34]. Future studies should include functional assays of T cells to further discern these T cells as activated or exhausted/ senescent. In addition, immune changes specific to episodes of ACR in the first 6–12 months post-transplant should be characterized given that the ACR episodes captured in our study cohort occurred 1–11 years post-transplant.

There are several limitations to our study. First, although we studied a large single center cohort of ITx patients, we were limited overall by our cross-sectional approach and small sample sizes across clinical states. Second, we excluded samples that met criteria for both infectious enteritis and ACR simultaneously. Concomitant ACR and infectious enteritis is an important etiology of allograft dysfunction [5,35,36], which may be due to immune activation during infection precipitating a rejection episode, decreased absorption of immunosuppression medication during infection leading to rejection, or infection following additional immunosuppression for rejection. In addition, we did not differentiate between bacterial and viral enteritis, which are likely characterized by distinct immune responses to bacterial and viral pathogens. Arguably viral enteritis may be more common in patients who are more immunosuppressed or leukopenic. Also, we are unable to control for the effect of confounding factors including immunosuppression and liver-inclusive allograft in this small cohort of patients. These factors may in fact contribute to the differences in samples collected late versus early post-ITx that we observed in this group of patients. Moreover, our normal samples were defined based on clinical symptoms without endoscopic evidence of normal allograft function due to the frequency of our peripheral blood collections and the inability to time blood sample collection with surveillance endoscopies. Lastly, our intestinal failure controls were dissimilar from our ITx recipients in terms of age and underlying intestinal failure diagnosis which may account for differences observed between the groups. Another future control group would include age-matched healthy controls, given that intestinal transplant recipients now no longer have intestinal failure. A multi-center study with larger cohorts and a prospective study design would be essential to address all of these limitations.

Our study has laid the groundwork for a number of future studies to further investigate the immune mechanisms of allograft dysfunction after ITx. To assess the role of these T cell markers as a potential biomarker for allograft dysfunction, it is critical that we elucidate the

presence of T cells in allograft tissue across clinical states and over time. It would also be valuable to expand T cell immunophenotyping to other causes of allograft dysfunction following ITx, such as antibody-mediated injury [37], chronic rejection [38], and post-ITx inflammatory bowel disease-like inflammation [39]. A recent study suggests that bidirectional intragraft alloreactivity may affect clinical outcome after ITx [40]. Additional studies may also consider evaluating T cell chimerism among these clinical states of allograft dysfunction. In the setting of chimerism, donor-derived cells may affect immunophenotypes. Therefore donor characteristics such as age may prove to be important clinical factors. Likewise, future studies should also further assess the changes in T cells associated with immunosuppression use using multivariable models as well as evaluate less commonly used agents such as infliximab and alemtuzumab. A more in depth understanding of the effect of immunosuppression on T cell immunophenotypes will enhance the clinician's ability to select the appropriate immunosuppression regimen for each individual patient.

In conclusion, in this pilot study, the immunophenotype of CD4 T cells is enriched with more central memory cells in samples collected late versus early after ITx. We also observed an increase in activation markers of CD4 and CD8 T cells in ACR. Additional studies are needed with larger cohorts to validate these changes in the T cell immunophenotype. Further elucidating T cell immunophenotypes in ITx will lead to a better understanding of immune mechanisms of allograft dysfunction, identification of potential biomarkers in ITx, and optimized selection of immunosuppressive therapies.

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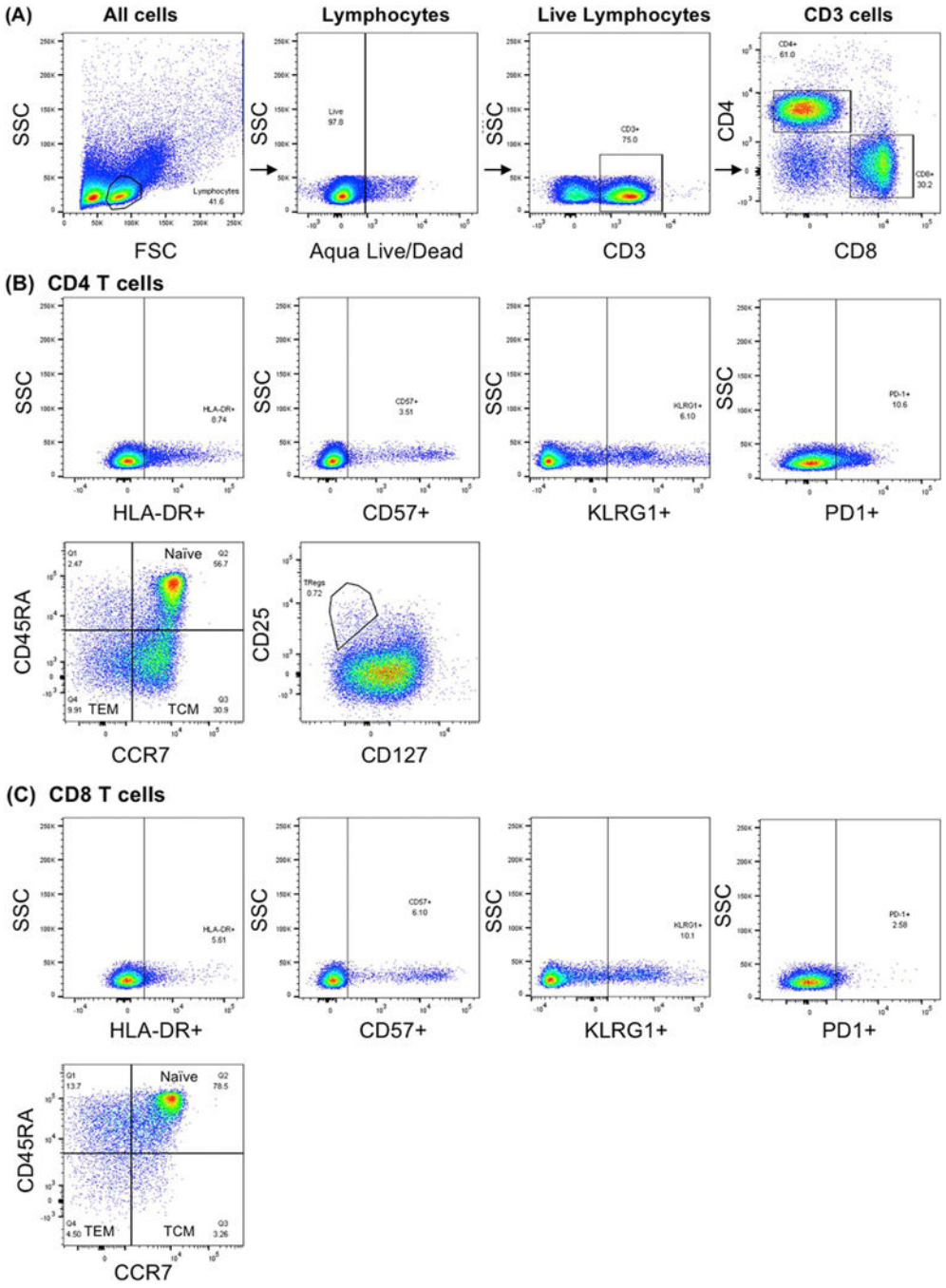

Fig. 1.

T cell immunophenotyping strategy. Scatterplots of flow cytometry data demonstrating the gating strategy to identify: **(A)** CD4 and CD8 T cell subsets. **(B)** Percentage of CD4 T cells with expression of activation markers, naïve/memory CD4 T cells, and T regulatory cells. **(C)** Percentage of CD8 T cells with expression of activation markers and naïve/memory CD8 T cells.

TCM: T central memory cells; TEM: T effector memory cells; TRegs: T regulatory cells.

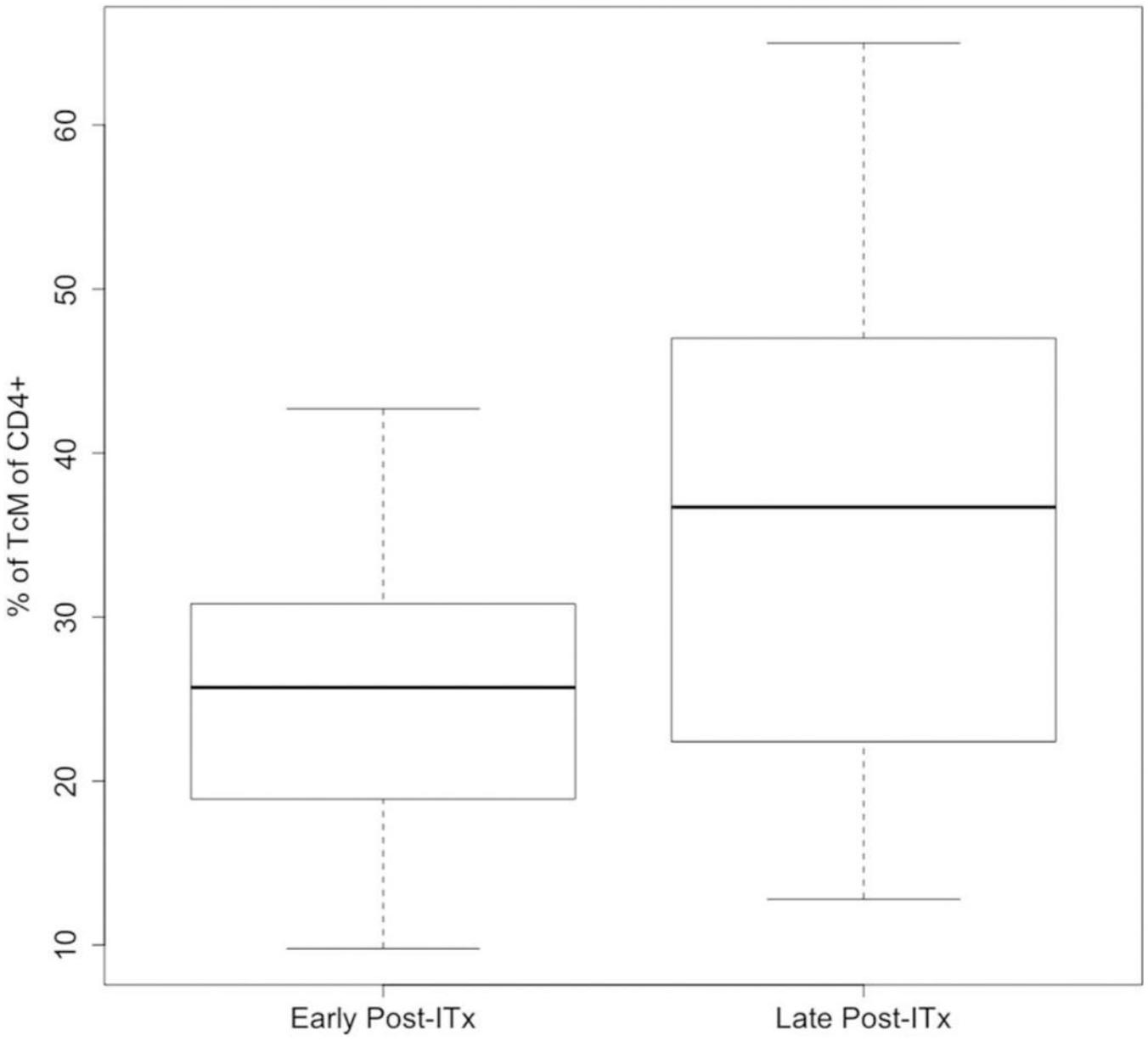

Fig. 2.

Box and whisker diagram of CD4 T central memory cells in the early post-ITx samples and late post-ITx samples. Lower and upper box borders indicate 25th and 75th percentiles, respectively. Lines within each box indicate median percentage of CD4 central memory cells. Whiskers above and below each box indicate maximum and minimum values, respectively. Difference between early and late post-ITx samples: 11.4; 95% CI (4.7, 18.2); p=0.001

Table 1

Antibody-fluorochrome panel.

Fluorochrome	Marker
FITC	CD57
PE	KLRG1
PE CY7	PD-1
APC	CCR7
Alexa 700	CD8
APC Cy7	CD3
BV510	LIVE/DEAD
BV570	CD4
BV650	HLADR
BV785	CD45RA

Table 2

Clinical characteristics of study population.

Demographic	Intestinal transplant	Intestinal failure	*p*-Value
	***N* = 31**	***N* = 7**	
Retransplant, n (%)	5 (16)	–	–
Median age at the time of ITx in years (IQR) (years)	3 (1.0–9.0)	–	–
Liver-inclusive allograft, n (%)	27 (87)	–	–
Pediatric (< 21 yrs), n (%)	26 (84)	7 (100)	0.56
Median age at study enrollment (IQR) (years)	11.5 (8.1–16.7)	9.9 (6.9–10.6)	0.21
Male, n (%)	20 (63)	5 (71)	> 0.90
Primary etiology of intestinal failure, n (%)			
• Surgical short bowel syndrome	25 (81)	2 (29)	**0.02**
• Motility disorder	2 (6)	5 (71)	
• Congenital enterocyte disorder	2 (6)	0	
• Other	2 (6)	0	
Ethnicity, n (%)			
• Hispanic	18 (58)	6 (86)	0.2
• Caucasian	9 (29)	0	
• Other	4 (13)	1 (14)	

The bold indicates statistical significance.

Table 3

Differences in percentage of naïve and memory T cells in late (n = 32) versus early (n = 19) post-ITx samples.

	Difference between Late vs Early Post-ITx samples (95% CI)	*p*-Value	FDR-adjusted *p*-value
% of CD4 cells			
Naïve	−5.7 (−15.0, 3.7)	0.24	0.51
Central memory	11.4 (4.7, 18.2)	**0.001**	**0.03**
Effector memory	−4.4 (−9.7, 0.9)	0.11	0.51
% of CD8 cells			
Naïve	8.7 (−3.8, 21.3)	0.17	0.51
Central memory	0.5 (−0.7, 1.7)	0.39	0.61
Effector memory	−2.4 (−5.2, 0.3)	0.09	0.51

The bold indicates statistical significance.

Table 4

Differences in percentage of naïve and memory T cells in normal samples based on immunosuppression.

	Corticosteroid versus no Corticosteroid			Sirolimus versus no Sirolimus			MMF versus no MMF		
	Difference (95% CI)	***p*-Value**	**FDR-adjusted *p*-value**	**Difference (95% CI)**	***p*-Value**	**FDR-adjusted *p*-value**	**Difference (95% CI)**	***p*-Value**	**FDR-adjusted *p*-value**
% of CD4									
Naïve	0.7 (−7.6, 9.0)	0.87	0.90	−2.1 (−13.6, 9.3)	0.72	0.91	10.3 (1.5, 19.2)	**0.02**	0.62
Central memory	−6.0 (−12.5, 0.5)	0.07	0.64	1.1 (−7.6, 9.7)	0.81	0.91	−6.5 (−13.7, 0.8)	0.08	0.76
Effector memory	3.2 (−1.9, 8.2)	0.22	0.79	−0.7 (−6.5, 5.0)	0.80	0.91	1.4 (−4.1, 6.9)	0.62	0.98
% of CD8									
Naïve	2.5 (−9.6, 14.5)	0.69	0.90	−10.3 (−23.4, 2.8)	0.13	0.70	6.9 (−5.9, 19.7)	0.29	0.82
Central memory	−0.5 (−1.6, 0.6)	0.40	0.85	−1.2 (−2.6, 0.1)	0.06	0.55	0.0 (−1.2, 1.3)	0.95	0.98
Effector memory	−1.4 (−3.8, 1.0)	0.25	0.79	0.4 (−3.3, 4.0)	0.84	0.91	−0.6 (−3.5, 2.3)	0.68	0.98

MMF: Mycophenolate mofetil.

The bold indicates statistical significance.

Table 5

Differences in percentage of naïve and memory T cells in ITx (n = 51) versus intestinal failure (n = 7) samples.

	Difference (95% CI)	*p*-Value	FDR-adjusted *p*-value
% of CD4 cells			
Naïve	−15.6 (−29.9, −1.3)	**0.03**	0.09
Central memory	2.3 (−9.0, 13.6)	0.69	0.83
Effector memory	10.9 (3.9, 18.0)	**0.002**	**0.008**
% of CD8 cells			
Naïve	6.3 (−10.8, 23.5)	0.47	0.66
Central memory	0.5 (−1.2, 2.1)	0.57	0.73
Effector memory	−4.3 (−9.0, 0.4)	0.07	0.17

The bold indicates statistical significance.

Table 6

Differences in T cell activation markers in ACR versus infectious enteritis, ACR versus normal, & infectious enteritis versus normal samples.

	ACR (n=5) versus infectious enteritis (n=14)			ACR (n=5) versus normal (n=51)			Infectious enteritis (n=14) versus normal (n=51)		
	Difference (95% CI)	***p*-Value**	**FDR-adjusted *p*-value**	**Difference (95% CI)**	***p*-Value**	**FDR-adjusted *p*-value**	**Difference (95% CI)**	***p*-Value**	**FDR-adjusted *p*-value**
% of CD4									
HLA-DR+	5.8 (−3.4, 15.0)	0.22	0.37	8.2 (2.5,13.9)	**0.005**	0.05	2.5 (−0.2, 5.2)	0.07	0.75
CD57+	8.1 (0.9, 15.3)	**0.03**	0.09	7.8 (3.2, 12.4)	**0.001**	**0.01**	−0.8 (−2.9, 1.3)	0.45	0.89
KLRG1+	5.0 (−4.0,14.1)	0.28	0.43	4.4 (−1.7, 10.5)	0.15	0.29	0.4 (−3.0, 3.9)	0.80	0.93
PD1+	3.5 (−7.5, 14.5)	0.53	0.65	6.6 (−1.6,14.8)	0.11	0.25	1.1 (−2.9, 5.2)	0.59	0.93
% of CD8									
HLA-DR+	23.5 (8.5, 38.6)	**0.002**	**0.009**	21.0 (10.8, 31.2)	**< 0.001**	**< 0.001**	1.0 (−3.9, 5.9)	0.69	0.93
CD57+	26.0 (15.1, 36.8)	**< 0.001**	**< 0.001**	18.0 (3.0, 33.0)	**0.02**	0.08	−5.5 (−13.9, 2.9)	0.20	0.82
KLRG1+	41.4 (26.1, 56.6)	**< 0.001**	**< 0.001**	21.5 (5.0, 38.0)	**0.01**	0.07	−5.4 (−15.1, 4.3)	0.27	0.82
PD1+	4.4 (−2.0, 10.8)	0.18	0.37	5.6 (1.3, 10.0)	**0.01**	0.07	0.3 (−1.6, 2.3)	0.75	0.93

The bold indicates statistical significance.