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Relationship Between Antithymocyte Globulin, T Cell Phenotypes and Clinical Outcomes in Pediatric Kidney Transplantation

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Abstract

Depletional induction using anti-thymocyte globulin (ATG) reduces rates of acute rejection in adult kidney transplant recipients, yet little is known about its effects in children. Using a longitudinal cohort of 103 patients in the Immune Development in Pediatric Transplant (IMPACT) study, we compared T cell phenotypes after ATG or non-ATG induction. We examined the effects of ATG on the early clinical outcomes of alloimmune events (development of *de novo* donor specific antibody and/or biopsy proven rejection) and infection events (viremia/viral infections). Long-term patient and graft outcomes were examined using the Scientific Registry of Transplant Recipients. After ATG induction, although absolute counts of CD4 and CD8 T cells were lower, patients had higher percentages of CD4 and CD8 memory T cells with a concomitant decrease in frequency of naïve T cells compared to non-ATG induction. In adjusted and unadjusted models, ATG induction was associated with increased early event-free survival, with no difference in long-

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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term patient or allograft survival. Decreased CD4+ naïve and increased CD4+ effector memory T cell frequencies were associated with improved clinical outcomes. Though immunologic parameters are drastically altered with ATG induction, long term clinical benefits remain unclear in pediatric patients.

1. Introduction

Kidney transplantation is the optimal treatment for end stage renal disease, providing children with improved outcomes compared to dialysis[1–3]. Despite advances in care, immunosuppression causes significant morbidity in pediatric transplant patients including risk for infection while only providing partial protection against allograft rejection[4]. Registry data from multiple sources demonstrate that infection and rejection remain a significant concern in the first year post-operatively as this may impact long-term clinical outcomes[5–8] In the Immune Development in Pediatric Transplantation (IMPACT) trial[9], we demonstrated that 24% of pediatric kidney transplant recipients developed allograft rejection, while over 60% suffered from viremia and/or viral infection in the first year after transplant. There is an urgent need to improve our understanding of the immunological effects of current immunosuppressive regimens in order to tailor treatment. This is especially true for depletional induction agents as their immunomodulatory impact can be profound and their clinical benefit in children remains unclear[10–12].

Greater than 90% of US children receive induction therapy. Depletional induction with anti-thymocyte globulin (ATG) is used more often than IL-2 receptor blockade (70% versus 30%)[6] to reduce the rate of acute rejection and delayed allograft function[13]. However, ATG has been associated with an increased risk of infectious complications in adult kidney transplant recipients[14,15]. By contrast, the use of ATG in pediatric kidney transplant patients has been paradoxically associated with fewer infections[16], although the mechanism remains elusive. The T cell repertoire after ATG induction is known to have a higher percentage of memory T cells, yet granular longitudinal data in children remain scant[17]. An understanding of how ATG affects T cell populations over time and the association of these immunologic changes with short and long-term clinical outcomes would provide valuable insight into the appropriate use of ATG in pediatric transplantation.

In the present study, we examined the relationships between ATG induction, T cell phenotypes, and short and long-term clinical outcomes in a longitudinal, observational study of pediatric kidney transplant recipients. We sought to understand the effect of ATG on T cell populations over time. We subsequently determined the incidence of acute rejection and/or the formation of de novo DSA (*dn*DSA), and the incidence of viral infection in relation to induction type as well as long-term patient and allograft survival.

2. Methods

2. 1 Patients and Study Design

We performed secondary analyses utilizing the IMPACT study, a multi-center prospective observational trial (NCT00951353) in pediatric kidney transplant recipients age 1–20 years

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during the first year of kidney transplantation, as previously described[9]. Patients were enrolled from 7/2009–3/2012 and followed for one year after enrollment. The protocol was reviewed and approved by each sites' Institutional Review Board. Upon enrollment, baseline demographics were obtained. Immunosuppressive regimens included ATG and/or IL2 receptor blockade induction and prednisone-free or prednisone-based maintenance immunosuppression with a calcineurin inhibitor and anti-metabolite. Regimens were clinically managed according to site preference and patients were not randomized to specific therapies[9]. Of the 125 patients enrolled, 106 underwent transplant. Two of these 106 patients were excluded due to concomitant endpoints (both infection and alloimmune event) and one was excluded due to technical failure yielding a total of 103 patients in the analysis.

This study used data from the Scientific Registry of Transplant Recipients (SRTR). The SRTR data system includes data on all donor, wait-listed candidates, and transplant recipients in the US, submitted by the members of the Organ Procurement and Transplantation Network (OPTN). The Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services provides oversight to the activities of the OPTN and SRTR contractors. Long term allograft survival, patient survival, and development of post-transplant lymphoproliferative disorder (PTLD) were ascertained by merging our dataset with the SRTR using date of transplant, age, sex, and transplant center to match patients. For patients in which this combination of variables was not unique, we utilized human leukocyte antigen (HLA) to resolve ambiguity.

2.2 Infection Monitoring

An infection event was defined as viremia—with or without clinical symptoms—by polymerase chain reaction (PCR) for Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), adenovirus, JC virus, and human herpesvirus 6 (HHV6), HHV7, and HHV8 at pretransplant and at 1, 3, 6, 9, and 12 months after kidney transplantation (Viracor Eurofins Clinical Diagnostics, Lee's Summit, MO). PCRs were performed at a central lab and results of greater than or equal to 100 copies/mL were considered positive. Management of infections was performed according to clinical standard of each site[9].

2.3 Alloimmune Monitoring

An alloimmune event was defined as the presence of biopsy proven rejection including borderline rejection and/or the formation of *dn*DSA. Patients underwent biopsies per protocol at 6 and 12 months, and for clinical indication. Renal biopsies were graded using the Banff criteria by a central pathologist[18]. HLA- typing for the HLA-A, -B, -DRB1, -DRB3/4/5, and -DQB1 loci was performed using molecular methods. Additional typing for HLA-C, -DQA1,-DPA1, and -DPB1 was performed to assign *dn*DSA. Luminex single-antigen bead assay for detection of Class I and II specific antibodies was monitored at baseline, 1, 3, 6, 9, and 12 months (One Lambda, Canoga Park, CA). HLA antibodies were considered positive if mean fluorescence intensity was greater than 1000, except for HLA-Cw where the threshold was 2000[19]. These thresholds were chosen based our previous experience[20].

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2.4 Flow Cytometry

Peripheral blood was obtained pre-transplant, and at 1,3,6,9, and 12 months for flow cytometry. CD4+ and CD8+ T cells were analyzed for traditional memory subsets including naïve (Tnaïve, CD45RA+CCR7+), central memory (Tcm, CD45RA-CCR7+), effector memory (Tem, CD45RA-CCR7-), and effector memory RA (Temra, CD45RA+CCR7-). Samples were also stained for markers of senescence/exhaustion, CD57 and PD-1. T regulatory (Treg) cells were assessed on the basis of CD4+CD25+CD127- expression (Supplemental Figure 1). All flow cytometry was performed at a centralized laboratory in a standardized fashion to minimize variability.

2.5 Statistical Analysis

Because patients were not randomized to specific induction therapy, we assessed baseline characteristics between ATG and non-ATG groups using Wilcoxon rank-sum tests for continuous variables and chi-square tests or Fisher's exact test for categorical variables. To address treatment selection bias, we accounted for prednisone use as a covariate in all regression analyses. We also performed 1:1 propensity score matching without replacement using 0.25 standard deviation of linear propensity scores as the caliper as a sensitivity analysis. A logistic regression was fit to estimate the probability of receiving ATG (propensity score) using age, sex, race, ethnicity, panel reactive antibody (PRA), HLA A/B and DR mismatch, CMV serostatus, EBV serostatus, and prednisone use as covariates. Matching excluded more than 50% of the patients from the analysis, and thus was considered as a sensitivity analysis, not a main analysis. Variable ratio matching and inverse probability treatment weighting were also attempted but we were unable to account for an imbalance in confounders.

To investigate the effect of ATG on T cell phenotypes, frequencies and absolute counts of T cell compartments were plotted over time and stratified by ATG induction. Linear regression models using generalized estimating equations to account for within-subject correlation were fit to examine the effect of ATG on T cell phenotype over time. These models included ATG, time, interaction between time and ATG, and prednisone use as covariates. Interaction terms were removed from the model if not significant. To protect against multiple comparisons, pairwise comparisons between time points were made only if the overall omnibus test was statistically significant.

The primary clinical outcome was the first clinical event after transplantation, defined as either an alloimmune event or an infection event. Patients were followed from the time of transplantation until an alloimmune or infection event, or 1-year post-transplantation, whichever occurred first. The alloimmune and infection events were considered as competing risks because the occurrence of one event altered the risk of having another event. The cumulative incidences of the alloimmune and infection events were plotted and compared between ATG and non-ATG groups at 1-year post-transplantation using Gray's test and Fine-Gray sub-distribution hazards models which adjusted for prednisone use. In addition, event-free survival, defined as time from transplant to any event (either alloimmune or infection), whichever occurred first, was also considered as a secondary outcome. Event-free survival was estimated by the Kaplan-Meier method and was compared between ATG

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and non-ATG groups using log-rank test and Cox proportional hazards adjusted for prednisone use.

We ascertained long-term patient and graft survival in both our original cohort and our propensity matched cohort using SRTR data. Seven-year post-transplant survival was estimated by the Kaplan-Meier method and compared between ATG and non-ATG groups by log-rank tests.

Finally, T cell phenotype at baseline, 1 and, 3 months were compared between clinical outcome groups (infection vs. alloimmune vs. no event) using a Kruskal-Wallis test to determine if the distributions of cellular phenotypes were different between the groups. Cellular phenotypes measured after an event (either infection or alloimmune) were excluded from the analysis.

All statistical tests used a two-sided significance level of 0.05 without adjusting for multiplicity. All analyses were performed using R 3.5.1 (R Core Team, Vienna, Austria) and SAS 9.4 (SAS Institute, Cary, NC).

3. Results

3.1 Baseline Characteristics

Patient characteristics were compared between ATG and non-ATG groups (Table 1). Groups were similar in age, sex, ethnicity, sensitization status by PRA, HLA matching, etiology of disease, pre-existing DSA, CMV serostatus, EBV serostatus, and EBV/CMV mismatch. There were more African-Americans (16% vs. 4%) and an increased use of both prednisone (70% vs. 31%) and sirolimus (23% vs. 4%) in the non-ATG group. There were also differences in use of ATG by site, with Center 1 not using ATG at all and Center 2 accounting for 81% of patients who received ATG.

3.2 ATG and T Cell Memory Phenotypes

We first examined the proportion of T cell memory phenotypes over time in relation to induction type. The proportion of CD4+ naïve cells (Figure 1a) was similar between groups at baseline and lower with ATG thereafter (mean difference at 1 month −21.7%, 95%CI: −35.5%,−7.9%). The proportion of CD4+ Tem (Figure 1b) was also similar at baseline and higher in the ATG group thereafter (mean difference at 1 month 17.4%, 95%CI:7.2%,27.6%). CD8+ naïve frequencies trended lower after ATG induction compared to non-ATG induction (mean difference −7.5%, 95%CI:−15.7%,0.6%, Figure 1e). The proportion of CD8+ Tcm was higher after ATG induction, though this difference was also present at baseline (mean difference 3.4%, 95%CI:1.5%,5.3%, Figure 1g).

We saw a marked decrease in the absolute number of CD4+ and CD8+ T cells across the majority of T cell phenotypes among patients with ATG induction compared with those without ATG induction over time (all $p<0.05$). However, CD8+ Tem did not differ between ATG and non-ATG groups (Supplemental Figure 2). In a propensity score matched cohort, similar patterns were demonstrated for both cell frequency and absolute cell counts (Supplemental Figure 3). There did not appear to be a differential depletion of T cell

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phenotypes between naïve and memory populations at 1-month post-depletion (Supplemental Figure 4).

3.3 ATG and T Cell Markers of Terminal Differentiation

We next examined terminally differentiated CD4 and CD8 T cells. The frequencies of CD4+CD57-PD1+ (mean difference at 1 month 12.9%, 95%CI:5.4%,20.4%) and CD4+CD57+PD1+ T cells were higher after ATG induction (mean difference at 1 month 2.9%, 95% CI:0.9%,4.9%, Figure 2a–b). The frequency of CD4+CD57-PD1- T cells was lower after ATG induction (mean difference at 1 month −16.4%, 95%CI:−25.6%,−7.1%, Figure 2d). Among CD8+ T cells, CD8+CD57-PD1+ T cell frequencies trended higher 6-months post-transplant compared to non-ATG groups (mean difference at 6 months 7.7%, 95%CI:−1.5%,17.0%, Figure 2e). Additionally, the point estimates for CD8+ CD57+ PD1- frequency were elevated after ATG at 12 months post-transplant (mean difference at 12 months 5.1%, 95%CI:−3.1%,13.2%, Figure 2g). Again, there were marked decreases in the absolute number of cells across these subsets with ATG ($p<0.05$ for all). Only CD8+ CD57+PD1+ and CD8+ CD57+PD1- cell subset counts did not differ by ATG usage (Supplemental Figure 2). Phenotypic analysis was unchanged in our propensity score matched cohort (Supplemental Figure 5).

3.4 ATG and Regulatory T Cells

A higher frequency of CD4+ Tregs was observed with ATG induction at 1- and 3-months post-transplantation (mean difference at 1 month 5.7%, 95%CI:3.4%,7.9%; at 3 months 2.3%, 95%CI:1.2%,3.4%). At 12 months, patients who underwent non-ATG induction trended towards a higher frequency of CD4+ Tregs (mean difference at 12 month −0.9%, 95%CI:−2.0%,0.1% Figure 3). In contrast, the total number of Tregs was lower in the ATG group at baseline and throughout the study (mean difference at 12 months −26.0, 95% CI: −35.8,−16.2, Supplemental Figure 6). This remained unchanged in the propensity matched cohort (Supplemental Figure 7).

3.5 ATG Induction and Clinical Outcomes

Each patient was assigned to one of three groups based on the first clinical event that occurred: alloimmune event, infection event, or no event. Overall, 85% (n=88) of the cohort experienced an event.

Fifteen percent of ATG groups and 23% of non-ATG had alloimmune events. One patient in the ATG group experienced ACR and one had borderline rejection. In the non-ATG group 4 patients experienced ACR and 7 had borderline rejection. Patients induced with ATG or non-ATG agents developed *dn*DSA at similar rates and received similar treatment for rejection (Table 2, Supplemental Table 2).

Sixty-nine percent of the non-ATG group and 50% of the ATG group experienced a viral infection, with EBV being the most common (Table 2).

Patients who underwent ATG induction had a higher event-free survival (ATG 34.6%, 95%CI 20.4%−58.7%; non-ATG 7.8%, 95%CI 3.6%−16.8%), than those who underwent

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non-ATG induction (log-rank test, p=0.01, Figure 4a). Given that prednisone usage was different between ATG and non-ATG groups, we fit Cox proportional hazards to adjust for prednisone use. In this analysis, the hazard of any clinical event was 45% lower with ATG compared to non-ATG induction (adjusted hazard ratio 0.55, 95%CI: 0.32–0.96, p=0.036).

There were no differences in the cumulative incidence of alloimmune events (Supplemental Figure 8a) or infection events (Supplemental Figure 8b) between ATG and non-ATG groups. No differences were seen in event-free survival or incidence of alloimmune/infection events in the propensity matched group (Supplemental Figure 9); however this analysis was considered a sensitivity analysis due to our inability to match a large proportion of the subjects.

There were no patient deaths or graft failures in the 1-year study period. When examining long term follow-up, for both the original cohort (Figure 4 b/c) and the propensity matched cohort (Supplemental Figure 10) there were no differences in patient or allograft survival over seven years of follow-up. Only one patient in the cohort (non-ATG group) developed PTLD 3.5 years after transplant and was treated successfully.

3.6 T Cell Phenotypes are associated with event free survival

We examined the association of T cellular phenotypes with clinical outcomes. A higher frequency of CD4+ Tem and a lower frequency of CD4+ Tnaïve were associated with a no event phenotype (Figure 5 a & b). Additionally, an increased frequency of CD4+ CD57+ PD1- T cells at baseline, 1 and 3 months was associated with a no event phenotype (Figure 5c).

4. Discussion

We utilized a well-characterized, longitudinal pediatric cohort and demonstrated that ATG induction was associated with profound and sustained changes in T cell phenotype. The absolute counts of most T cell subsets were significantly decreased after ATG induction compared to non-ATG induction. Additionally, pediatric recipients induced with ATG had a T cell repertoire which was more memory in nature than those not induced with ATG. The frequencies of CD4+ Tem and PD1+ CD4+ T cells were higher after ATG induction than with non-ATG induction. CD4+ Tnaïve frequency was lower with ATG induction than non-ATG. CD8+ T cells exhibited a similar pattern with a higher frequency of CD8+ Tcm and lower frequency of CD8+ Tnaïve cells with ATG compared to non-ATG induction. We demonstrated that ATG induction was associated with improved short-term clinical outcomes. However, there were no differences in long-term patient or graft survival. Although absolute counts of CD4+ T cells were lower after ATG induction, an increased percentage of CD4+ Tem and decreased percentage of CD4 Tnaïve were directly associated with early freedom infection and alloimmune events. While, the present study confirms the profound immunologic changes seen with ATG induction in adults are also present in children, long term clinical benefits remain uncertain.

Consistent with adult studies[17,–21], pediatric kidney transplant patients exhibited a higher frequency of mature, effector T cells after ATG induction. CD4+ Tem and CD8+ Tcm

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frequencies were higher with ATG than non-ATG regimens over the one-year follow-up period. A previous study in children showed polarization to more memory type T cell subtypes in both CD4 and CD8 compartments over a period of 6 months[17]. They showed that ATG induction led to efficient depletion of naïve CD4+ T cells with little effect on CD4+ Tem. We did not observe a differential depletion of memory and naïve T cells, a result which has been inconsistently reported in the literature[22–24]. However, absolute counts were higher among all subsets of naïve/memory T cells in the non-ATG group compared to the ATG group immediately after induction. This early lymphopenic period may increase risk of infection, however we did not observe this.

We also interrogated terminally differentiated T cells by expression of CD57 and PD1 and demonstrated a lower percentage of CD57-PD1- CD4+ T cells and a higher percentage of CD57-PD1+ CD4+ T cells after ATG induction compared to non-ATG regimens. This may be concordant with a more robust immune response to fight infections [25] that is especially important in children who have less mature immune systems[26]. Additionally, these more differentiated cells may partially mediate protection from infection during the early lymphopenic period. Moreover, recent work has shown an increase in the frequency of CD57-PD1+CD4+ T cells, a population we show is increased after ATG induction in pediatric patients, is associated with improved clinical outcomes in adults[27]. Interestingly, some patients in our study exhibited mature immune phenotypes even at baseline, a finding that has been previously described in pediatric patients with chronic kidney disease[28].

Tregs are immune regulatory cells and are thought to be important mediators of the reduced rejection seen with ATG in adults[29]. Similar to prior studies, we demonstrated that ATG was associated with a higher proportion (though lower absolute count) of Treg cells soon after transplantation [17,29]. While these cells have been associated with decreased acute rejection seen after ATG induction in adult trials, this phenomenon was not observed in the present study. Additionally, some data suggest that absolute number of Tregs at 1 year is correlated with long-term graft survival[30]. As increases in the proportion of Tregs with ATG in this study were early and transient, it may be that their impact was confined to early time points. As noted above, the absolute number of Tregs was lower with ATG induction which may diminish their effect; however, some have argued that the ratio of Tregs to effector T cell subsets is what is of importance[29].

As we saw differences in T cell subsets after ATG induction, we next investigated differences in clinical outcomes. In previous studies, ATG induction in pediatric patients was associated with decreased infectious complications in children [15]. We observed an increased short-term event-free survival (i.e. freedom from either alloimmune or infection events) in the ATG group, however, the hazard of neither alloimmune nor infection events was individually different between ATG and non-ATG groups. Although patients with ATG in our study exhibited fewer absolute alloimmune events, the proportions were not statistically different. Adult studies have shown an association between ATG and decreased rates of acute rejection[31]. These studies have also examined a composite endpoint of acute rejection, graft loss, death, or loss to follow-up and found superiority of ATG over time[31]. However, we did not find any differences in long term patient or graft survival in the present study.

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This may be due to the end points examined or the particular social, clinical, and immunologic differences between children and adults.

Previous studies have shown that pre-transplant levels of CD4+ recent thymic emigrants are associated with rejection after ATG induction[32]. As thymic output is much higher in children[33], this may be one reason, in addition to small sample size, why we did not demonstrate protection from alloimmune events in our cohort. Furthermore, multiple prior mechanistic studies have shown that peripheral T cells which repopulate after induction switch to a memory phenotype[34–35]. Bamoulid and colleagues found that lymphocyte exhaustion and an increase in memory T cells were related to thymic output in adults induced with ATG, and in turn, this was associated with improved outcomes, including decreased rejection[36]. Indeed, our T cell phenotypic data are consistent with these findings as an increase in CD4+ Tem cells as well as mature CD57+ PD1- CD4+ T cells were associated with freedom from a clinically significant alloimmune or infection event. Overall, we believe that that there may be short term clinical benefits to “maturing” a pediatric immune system via depletional induction.

Although our study provides insight into the T cell phenotypes and the clinical course after ATG induction, there are important limitations. Patients were not randomized by induction method; therefore, other unmeasured patient factors may be responsible for the observed clinical outcomes, specifically, treating institution may impact both short- and long-term outcomes in kidney transplantation. For our analyses of T cell subsets, we believe that institution may represent an instrumental variable—as it causes variation in the exposure (ATG usage) but does not have a direct effect on the outcome of interest (T cell subsets)—and therefore do not include it in our model. Previous studies have shown that inclusion of instrumental variables increases model bias and further distorts results[37]. We also performed sensitivity analyses that showed no systematic effect of institution on T cell subsets (data not shown). Additionally, we cannot completely exclude the effects of prednisone or sirolimus as mediators of clinical outcomes, as our propensity matching was unsuccessful and therefore only a sensitivity analysis. Race was unbalanced between groups, with more black patients in the non-ATG group, a potentially important discrepancy as black race has previously been identified as a risk factor for rejection[38]. We only examined ATG induction at the time of transplantation and therefore may not detect effects of ATG used for the treatment of acute rejection. Finally, we do not find differences in long term outcomes with ATG induction as shown in adults. This may be due to the retrospective nature of our study, the endpoint used, or unmeasured factors that influence these outcomes. Regardless of these limitations, we believe that the observed differences between patients with ATG vs non-ATG induction are still informative.

In conclusion, ATG induction and subsequent immunosuppressive management in pediatric patients were associated with memory and mature T cell phenotypes. We also observed an increased event free survival within the first year but no difference in long-term outcomes with ATG induction. Though ATG has profound effects on immunologic parameters, a randomized study is needed to definitively ascertain the long-term clinical effects of ATG induction in pediatric patients to guide appropriate use.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ACR	Acute Cellular Rejection
ATG	Anti-thymocyte Globulin
CMV	Cytomegalovirus
***dn*DSA**	De-Novo Donor Specific Antibody
DSA	Donor Specific Antibody
EBV	Epstein-Barr Virus
HHV	Human Herpes Virus
HLA	Human Leukocyte Antigen
IMPACT	Immune Development in Pediatric Transplantation
PCR	Polymerase Chain Reaction
PRA	Percent Reactive Antibody
PTLD	Post-transplant lymphoproliferative disorder
RTE	Recent Thymic Emigrant
SRTR	Scientific Registry of Transplant Recipients
Tcm	T Central Memory cells
Tem	T Effector Memory cells

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Temra	T Effector Memory RA+ cells
Tnaïve	T naïve cells

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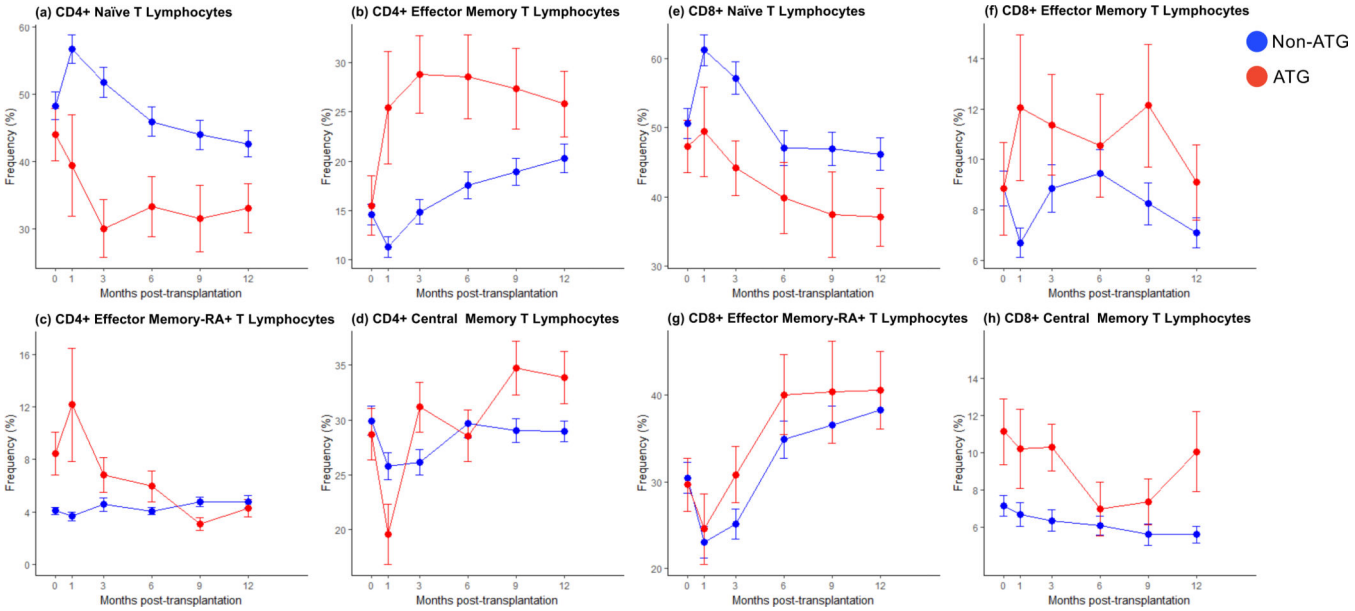

Figure 1: Frequency of CD4/8 T cell memory subsets by ATG induction

(a) CD4+ Tnaïve frequencies were lower with ATG induction and this difference varies with time ($p<0.0001$). (b) CD4+ Tem frequencies were higher with ATG induction and this difference varies significantly with time ($p=0.0002$). (c) CD4+ Temra frequency in ATG was higher at baseline than non-ATG induction at baseline and one month only ($p=0.0002$). (d) CD4+ Tcm frequency when comparing by ATG was higher with ATG at 3 and 9 months ($p=0.0001$). (e) CD8+ Tnaïve frequency did not vary by ATG induction ($p=0.07$). (f) CD8+ Tem frequencies did not vary by ATG induction ($p=0.24$) (g) CD8+ Temra frequency did not vary significantly with ATG induction ($p=0.53$). (h) CD8+ Tcm frequency was significantly higher with ATG induction though this difference was present at baseline ($p=0.0004$). For panels a-d, all p-values are Wald statistics for interaction between ATG and time in Type 3 GEE analysis. For panels e-h all p-values were Wald statistics for main effect of ATG in Type 3 GEE analysis because interactions between ATG and time were not significant.

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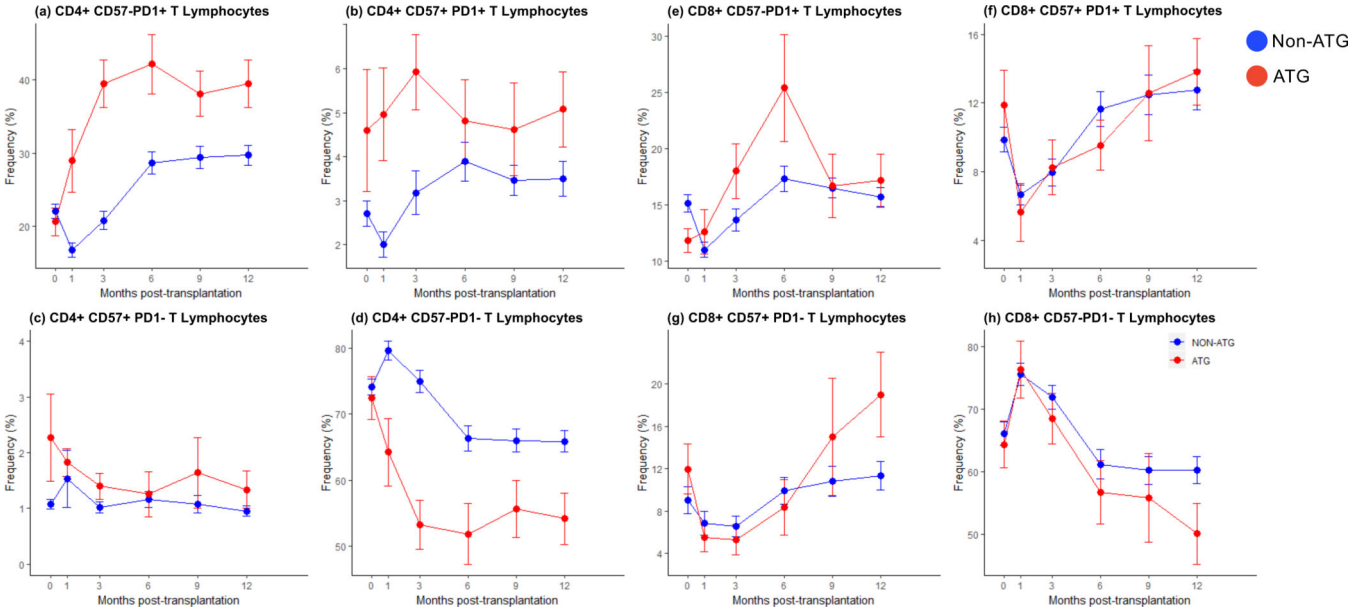

Figure 2: Frequency of CD4/8 T cell CD57/PD1 terminal differentiation subsets by ATG induction

(a) CD4+CD57- PD1+ frequency was significantly higher with ATG induction after baseline ($p<0.0001$). (b) CD4+CD57+ PD1+ frequencies did vary with ATG induction and the magnitude of this difference varied with time ($p<0.0001$) (c) CD4+CD57+ PD1- frequency did not vary significantly with ATG induction ($p=0.14$). (d) CD4+CD57- PD1- frequency was significantly lower with ATG induction and this varied with time, becoming significant after baseline ($p<0.0001$). (e) CD8+CD57- PD1+ frequency was higher with ATG induction at 3 and 6 months ($p=0.0238$). (f) CD8+ CD57+ PD1+ frequencies did not vary with ATG induction ($p=0.83$) (g) CD8+ CD57+ PD1- frequency was higher with ATG at 9 and 12 months varied with time ($p=0.0256$). (h) CD8+ CD57- PD1- frequency did not vary significantly with ATG induction ($p=0.59$). All p-values were Wald statistics for interaction between ATG and time in Type 3 GEE analysis except for CD4+CD57+PD1-, CD8+CD57-PD1-, and CD8+ CD57+ PD1+. In these cases, the interaction was not significant for and the p-value was from the test of main effect of ATG.

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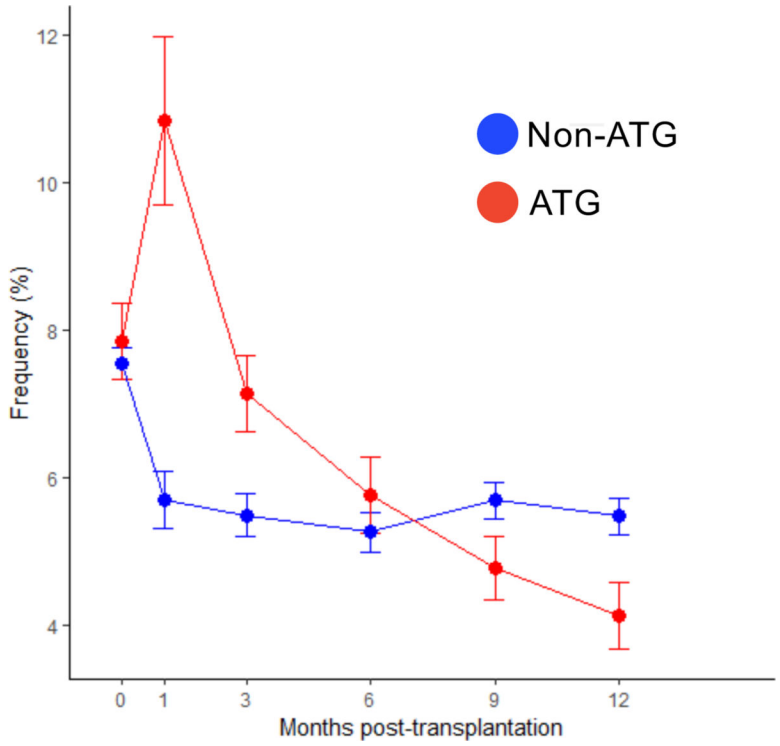

Figure 3: Frequency of CD4+ Treg

Treg frequency was significantly increased at 1 and 3 months after ATG induction compared with non-ATG induction ($p<0.0001$). P-value calculated from Wald statistic for interaction between ATG and time in Type 3 GEE analysis.

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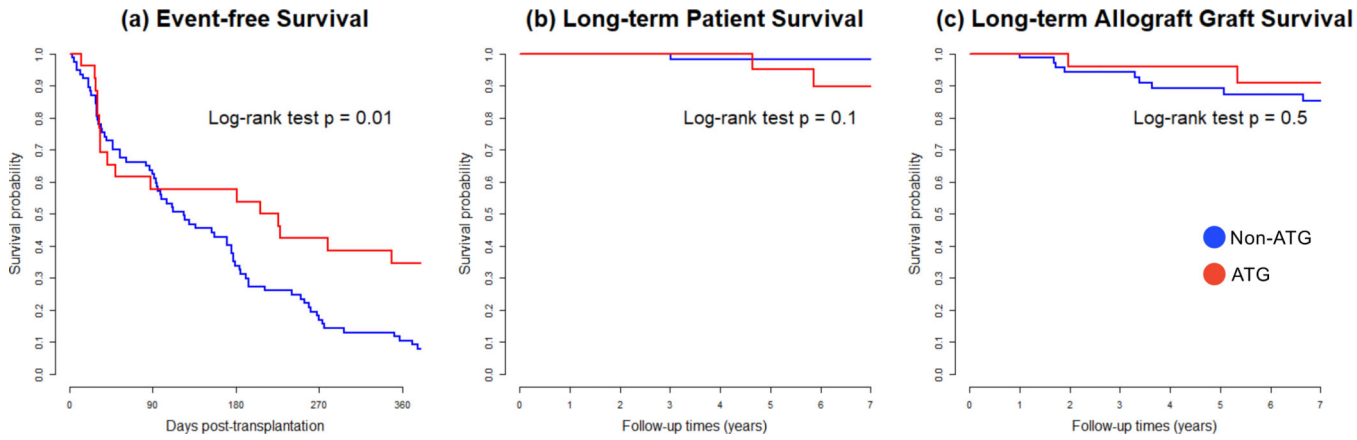

Figure 4: Post-transplant event analysis by ATG induction

(a) Event free survival(Log-Rank Test), (b) cumulative incidence of alloimmune events (Gray's Test), (c) cumulative incidence of infection events.

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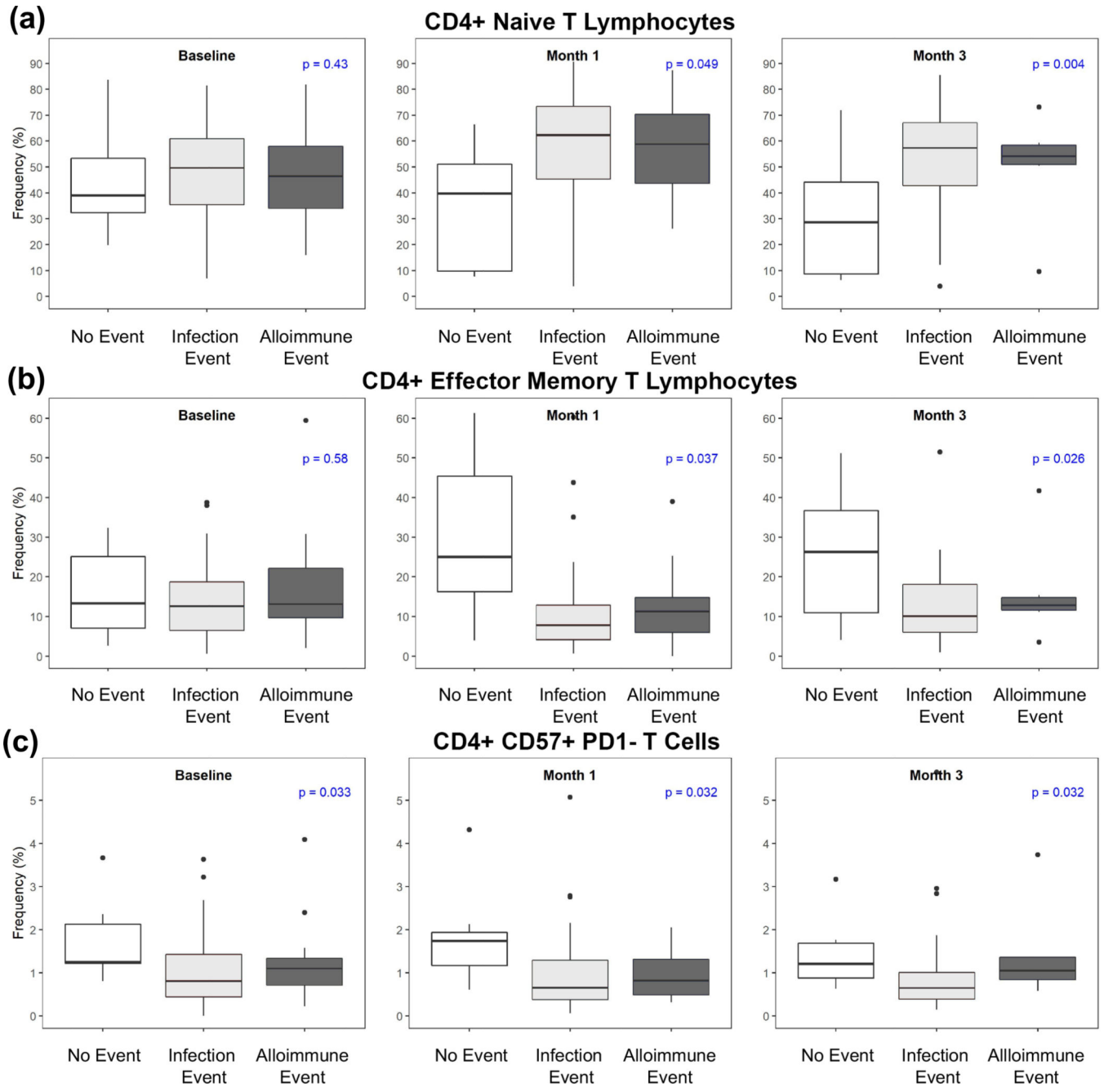

Figure 5: T Cell phenotypes associated with clinical events

(a) At 1 and 3 months, a decrease in CD4+ Tnaïve is associated with no event. (b) At 1 and 3 months, an increase in CD4+ Tem is associated with no event. (c) At baseline, 1 and 3 months CD57+PD1-CD4 T cells are associated with no event. All tests are Kruskal-Wallis without adjusting for multiple comparisons, $p<0.05$ significant.

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Table 1:

Demographics

	ATG N = 26	Non-ATG N = 77	P-Value
Age, Years Median (Q1-Q3)	16 (4–17)	14 (8–17)	0.56
Sex, Female N (%)	12 (46)	24 (31)	0.23
Race N (%)			**0.042**
Black or African American	1 (4)	12 (16)	
White	18 (69)	58 (75)	
Other Races	7 (27)	7 (9)	
Ethnicity –N (%)			0.82
Hispanic or Latino	11 (42)	35 (45)	
Not Hispanic or Latino	13 (50)	38 (49)	
Unknown	2 (7)	4 (5)	
Etiology			0.48
Cystic/Hereditary/Congenital	14(54)	48(62)	
Glomerulonephritis	7(27)	20(26)	
Hypertension	1(4)	0(0)	
Other	1(4)	2(3)	
Unknown	5(11)	7(9)	
Donor Type, Living N(%)	5(19)	21(27)	0.58
Panel Reactive Ab Present-N(%)	12(46)	22(29)	0.16
HLA Mismatch Median (Q1-Q3)	5 (3–5)	4 (3–5)	0.28
CMV Serostatus, Positive N (%)	14 (54)	36 (47)	0.65
EBV Serostatus, Positive N (%)	17 (65)	49 (63)	>0.99
CMV Mismatch (N%)	3(15)	12(29)	0.34
EBV Mismatch (N%)	7(31)	28(53)	0.13
Induction Medication N (%)			
Anti-Thymocyte Globulin	27(100)	0(0)	
Basiliximab	0 (0)	35 (45)	
Dacluzimab	2 (8)	42 (55)	
Maintenance Med-N(%)			
Prednisone	8 (31)	54 (70)	**0.0009**
Tacrolimus	26 (100)	76 (99)	>0.99
MMF	26 (100)	75 (94)	>0.99
Cyclosporine	0 (0)	2 (3)	>0.99
Azathioprine	3 (12)	4 (5)	0.36
Sirolimus	1 (4)	18 (23)	**0.038**
Leflunomide	0 (0)	1 (1)	>0.99
Pretransplant DSA-N (%)	3(3.9)	1(3.8)	>0.99
Site			**<0.0001**
Center 1	0(0)	32(41.6)	
Center 2	21(81)	11(14.3)	

	ATG N = 26	Non-ATG N = 77	P-Value
Center 3	5(19)	34(44.2)	

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Table 2:

Categorization of Events

Alloimmune Events by ATG Induction		
	Non-ATG (N = 77)	**ATG (N = 26)**
Alloimmune Event-n(%)	18(23)	4(15)
	Non-ATG Alloimmune Events(N=18)	ATG Alloimmune Events(N=4)
ACR 1A-n(%)	1(5.5)	0(0)
ACR 1B-n(%)	1(5.5)	1(25)
ACR 2A-n(%)	1(5.5)	0(0)
ACR 3-n(%)	1(5.5)	0(0)
Borderline-n(%)	7(39)	1(25)
Class I *dn*DSA-n(%)	0(0)	1(25)
Class II *dn*DSA-n(%)	7(39)	1(25)
Infection Events by ATG Type		
	Non-ATG (N = 77)	ATG (N = 26)
Infection Event-n(%)	53(69)	13(50)
	Non-ATG Infection Events (N=53)	ATG Infection Events (N=13)
BKV-n(%)	11(21)	1(8)
CMV-n(%)	6(11)	1(8)
CMV/other viral infection-n(%)	2(4)	0(0)
EBV-n(%)	18(34)	7(53)
EBV/BKV-n(%)	1(2)	0
EBV/other viral infection-n(%)	0(0)	1(8)
Other viral infection-n(%)	15(28)	3(23)

In total there were N=66 infections or 64% of the total cohort. In total, there were 22 alloimmune endpoints (21% of the total cohort). Grading based on Banff 2011 criteria. Abbreviations: BKV-BK Virus; EBV-Epstein-Barr Virus; CMV-Cytomegalovirus