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Risk factors for impaired CD4$^+$ T-cell reconstitution following rabbit antithymocyte globulin treatment in kidney transplantation

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Summary

To describe long-term CD4$^+$ T-cell reconstitution after rabbit antithymocyte globulin (rATG) treatment and identify predictive factors following kidney transplantation. A single-center retrospective study analyzed lymphocyte subsets in rATG-treated kidney transplant recipients (1986–2009). 589 patients were analyzed (maximum follow-up 21 years). A comparator group (n = 298) received an anti-IL-2 receptor monoclonal antibody. CD4$^+$ T-cell lymphopenia (<200/mm^3) was present in 48.5%, 9.2%, 6.7%,2.0%, and 0% of patients at one, three, five, 10, and 20 years post-transplant, respectively. CD4$^+$ T-cell count increased during the first 10 years but remained below the pretransplant count even after 20 years. At 1, 3, and 6 months post-transplant, mean CD4$^+$ T-cell count was significantly lower in patients with CD4$^+$ T-cell lymphopenia at 12 months versus patients without lymphopenia. On multivariate analyses, significant independent predictors for long-term impaired CD4 T-cell reconstitution were recipient age, pretransplant CD4$^+$ T-cell count, 12-month CD4$^+$ T-cell count, and tacrolimus or MMF therapy. Recipient age >40 years was identified as a cutoff point. CD4$^+$ T-cell reconstitution following rATG treatment remains impaired even after 21 years. Most risk factors for long-term impaired CD4$^+$ T-cell reconstitution may be evaluated pretransplant or are modifiable post-transplant.

Introduction

Rabbit antithymocyte globulin (rATG) is a lymphocyte-depleting preparation frequently used as induction therapy to reduce the risk of acute rejection following kidney transplantation. However, although rATG induction results in a low rate of rejection, reports from the early 2000s described an increased risk of opportunistic infections [1], malignancy [2–4], and mortality [1]. rATG-induced T-cell depletion is followed by immune reconstitution, with both new thymic emigration and homeostatic proliferation of memory T cells [5,6]. The rate of immune reconstitution is slow and highly variable, with some patients experiencing long-lasting CD4$^+$ T-cell lymphopenia [7]. The extent of CD4$^+$ T-cell lymphopenia appears to be a useful surrogate marker for malignancy [2,4] and mortality [8] and may be a clinically relevant parameter for the detection of overimmunosuppression. There are limited data to suggest that increasing age [9] and thymic function [6] are predictive of CD4$^+$ T-cell reconstitution up to 5 years after treatment with rATG [5,10]. No study, however, has examined potential risk factors for rATG-induced impaired CD4$^+$ T-cell reconstitution over a longer period. We report here a retrospective analysis of the kinetics of peripheral CD4$^+$ T cells

in a cohort of 589 kidney transplant recipients treated with rATG and analyze risk factors for impaired $CD4^+$ T-cell reconstitution, over a maximum follow-up period of more than 20 years. The objective of the analysis was to identify predictors of long-term impaired $CD4^+$ T-cell reconstitution in kidney transplant recipients following treatment with rATG treatment.

Patients and methods

Selection of patients

All patients who underwent kidney transplantation at the transplant unit of CHRU Tours, France, during 1986 to 2009 were included in this retrospective analysis if they received rATG during the first month post-transplant, either as induction therapy or to treat early steroid-resistant acute rejection, and if data on $CD4^+$ T-cell count were available.

Patients who received an anti-IL-2 receptor monoclonal antibody (anti-RIL-2 ab) during the same period were included in a comparator group if data on $CD4^+$ T-cell count were available.

Immunosuppressive treatment

The immunosuppressive regimen at our center changed throughout the study period. From 1986 to 1998, the majority of patients received rATG treatment (Thymoglobulin®, Genzyme Corporation, Cambridge MA, USA) as induction therapy unless they were at high risk of EBV infection (i.e. negative pretransplant EBV serology).

From 1998 onwards, rATG was mainly used for patients at increased immunological risk (defined as the presence of anti-HLA antibodies with panel reactive antibodies [PRA] >20%) and/or patients who received a kidney graft from an extended criteria donor. In the other cases, an anti-RIL-2 ab (basiliximab, Simulect®, Novartis Pharma AG, Basel Switzerland) was used. Induction therapy with rATG was started on the day of transplantation as a 12-hour infusion at a dose of 1.5 mg/kg. Subsequently, the dose was adapted to maintain a target blood $CD3^+$ T-cell count below 20 cells/mm^3. The duration of rATG administration varied between patients and treatment was discontinued when target calcineurin inhibitor concentrations were reached and/or when graft function had started.

The maintenance immunosuppressive regimen included cyclosporine or tacrolimus, mycophenolate mofetil (MMF) or azathioprine, and gradually tapered prednisone. Target trough levels at 3 months post-transplant were 150–250 ng/mL and 8–12 ng/mL for cyclosporine and tacrolimus, respectively [11]. The median dose of steroids was 10 mg/day at 3 months. Steroids were withdrawn during the first year following transplantation in patients with PRA <70%, no vasculitis or systemic lupus erythematosus, no history of acute cellular rejection, and no loss of a first graft due to immunological causes.

Follow-up and evaluation

All patients underwent regular clinical and biological evaluation, with at least a yearly checkup at the transplant center until death, end-stage renal disease, or retransplantation. Clinical and biological parameters were recorded at each consultation in the center's database.

The following pretransplant characteristics were recorded for all patients: age, gender, weight, type of renal disease, and history of any previous kidney transplant. After transplantation, information was collected regarding the duration and total dose of rATG treatment. Occurrences of acute rejection, cytomegalovirus (CMV) infection, and the type of immunosuppressive regimen at one year were also recorded.

All patients received trimethoprim-sulfamethoxazole during the first 3 months post-transplant. Patients at the highest risk of CMV disease/infection (D+/R-) received prophylaxis with a weekly infusion of intravenous immunoglobulin for 1 month (1986–1994), acyclovir (1995–2000), or ganciclovir (or valganciclovir) for 3 months (from 2001 onwards).

From 1986 to 1994, diagnosis of systemic CMV infection relied on optimized CMV culture on MRC5 human cells. CMV antigenemia was added in 1995 as a more rapid and direct evaluation of CMV viremia. Weekly CMV antigenemia monitoring was performed during the first 6 months following transplantation.

Lymphocyte count

A hemogram, including absolute lymphocyte count, was performed before transplantation and at each visit using a Coulter® LH 750 Hematology Analyzer (Beckman Coulter Inc, Brea CA, USA).

Lymphocyte T-cell subset counts were performed at baseline and annually. In a subpopulation of patients, lymphocyte T-cell subsets counts were also performed at 1, 3, and 6 months after transplantation. Blood samples were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-CD3 and phycoerythrin (PE)-conjugated anti-CD4 mAbs. Phenotypic analyses were performed according to a standard no-wash whole-blood procedure using a FACStar plus (Becton Dickinson, La Jolla, CA, USA) or an EPICS-XL-MCL flow cytometer (Beckman Coulter Inc, Brea CA, USA).

$CD4^+$ T-cell lymphopenia was defined as $CD4^+$ T-cell count $\leq 200/mm^3$. This cutoff value was defined *a posteriori*, as it has been reported to be associated with an increased

risk of opportunistic infections and cardiovascular disease [5,12].

Statistical analyses

Continuous variables are presented as mean ± standard deviation (SD), and categorical variables as percentages. Median values with interquartile range (IQR) are presented when the distribution of the parameters is not normal.

The associations between potential risk factors and long-term CD4$^+$ T-cell count after rATG treatment were examined by univariate and multivariate analysis using an adjusted generalized mixed-effects model. This model was selected because it can impute missing values and handle the highly correlated nature of repeated measurements within and between individuals [13]. Square root-transformed CD4$^+$ T-cell counts were used to approximate a normal distribution [14]. Analyses were performed using SAS 9.2 (SAS Institute Inc, Cary, NC, USA). P values ≤0.05 were considered to be significant for all analyses.

Results

Patient characteristics

Between 1986 and 2009, 589 patients were treated with rATG and provided at least one measurement of T-cell subsets during follow-up and were included in the analysis. The median duration of follow-up post-transplant was 9.8 years [IQR 5.2–14.9 years]. In total, 3332 values were used to estimate the curve (with a minimum of 1 point and a maximum of 10 points per patient). Baseline characteristics of the study population are shown in Table 1. The analysis population included 353 men and 236 women, with a mean age of 45.6 ± 14 years at time of transplant, and 100 patients received a second or third transplant. The median duration of rATG treatment was 8 days (IQR 6–11 days), and the median total dose of rATG was 6.8 mg/kg [IQR 4.9–10 mg/kg]. At 1 year post-transplant, 389 patients were receiving cyclosporine and 200 were receiving tacrolimus, 365 were receiving MMF, and 247 were receiving steroids. The percentage of patients receiving tacrolimus and MMF was 27% at 5 years post-transplant (versus 23% for cyclosporine and azathioprine), 10% at 10 years (versus 30%) and 10% (versus 43.6%) at 15 years.

From 1998, 298 patients were treated with an anti-RIL-2 ab and provided at least one measurement of T-cell subsets during follow-up and were included in the comparator group. This population included 187 men and 111 women, with a mean age of 48.2 ± 15 years. At 1 year post-transplant, 230 patients were receiving cyclosporine and 68 were receiving tacrolimus. All patients were receiving MMF and 143 were receiving steroids.

Immune reconstitution after ATG treatment

Absolute lymphocyte reconstitution

As shown in Fig. 1, the mean absolute lymphocyte count decreased after ATG treatment (1.53 ± 0.6 G/L pretransplant versus 0.93 ± 0.5 G/L at 1 year). The mean absolute lymphocyte count subsequently showed a slow increase, reaching a plateau after 5 years (1.27 ± 0.59 G/L at year 5 versus 1.38 ± 0.56 G/L at 20 years post-transplantation).

CD4$^+$ T-cell reconstitution after rATG treatment

The mean (± SD) pretransplant CD4$^+$ T-cell count was 782 ± 340/mm^3. After an initial depletion of CD4$^+$ T cells after the start of rATG treatment, the mean count increased rapidly during the first year after transplantation, reaching 235 ± 141/mm^3 at 1 year (Fig. 2a). Subsequently, it continued to increase, at a rate of 63/mm^3 per year between one and 5 years, and 41/mm^3 per year between five and

Table 1. Baseline characteristics of the analysis population (rATG) and the comparator group (anti-RIL-2 ab).

	Analysis population (rATG) N = 589	Comparator group (anti-RIL-2 ab) N = 298
Age (years), mean ± SD	45.6 ± 14	48.4 ± 15
Weight (kg) mean ± SD	67.5 ± 15.8	
Male, *n* (%)	353 (59.9)	185 (62.3)
Kidney disease, %		
Glomerulopathy	188 (31.9)	88 (29.8)
Polycystic kidney disease	89 (15.1)	53 (17.8)
Vascular	32 (5.4)	14 (4.9)
Interstitial tubular disease	56 (9.5)	31 (10.1)
Diabetic nephropathy	36 (6.1)	19 (6.4)
Other or unknown	188 (31.9)	93 (31)
Number of kidney transplants, 1/2/3, *n* (%)	489/88/12 (83.0/15.9/2.0)	292/6/0 (98/2/0)
Pretransplant cell count (/mm^3), mean ± SD		
Absolute lymphocyte	1530 ± 603	1579 ± 665
CD3$^+$ T cells	1163 ± 476	1187 ± 552
CD4$^+$ T cells	778 ± 337	799 ± 352
CD8$^+$ T cells	460 ± 227	444 ± 264
rATG treatment		
Duration (days), median [IQR]	8 [6–11]	–
Total dose of ATG (mg/kg), median [IQR]	6.8 [4.9–10]	–
Immunosuppressive regimen at 1 year (%)		
Steroids	247 (41.9)	
Cyclosporine	389 (66.0)	221 (74.5)
Tacrolimus	200 (34.0)	69 (23.2)
Mycophenolate mofetil	365 (62.0)	273 (91.7)
Azathioprine	224 (38.0)	4 (1.4)

SD, standard deviation, IQR, interquartile.

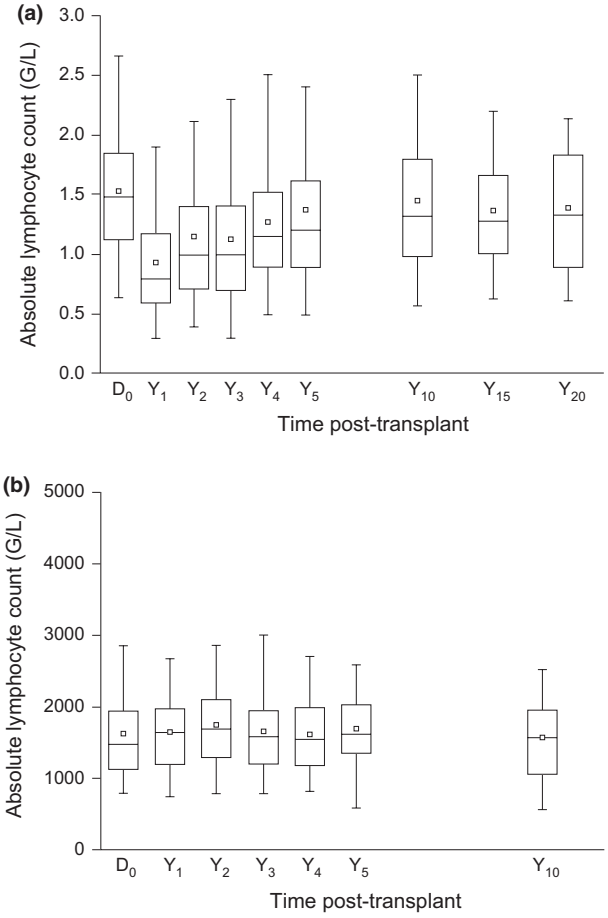

Figure 1 Box and whisker plot of absolute lymphocyte count over time post-transplant (a) after ATG treatment (b) in the comparator group receiving anti-RIL-2 ab. D, day; Y, year.

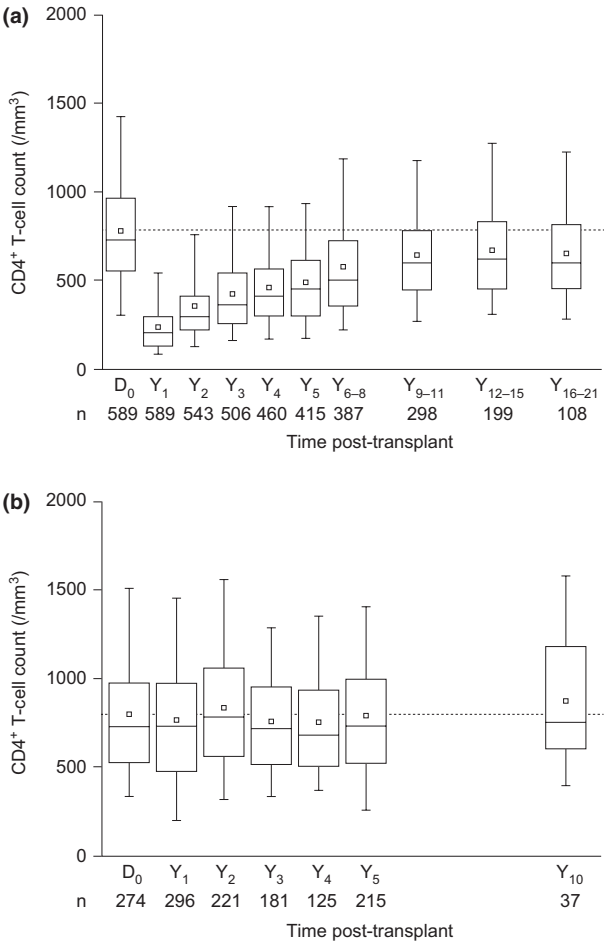

Figure 2 Box and whisker plots of CD4+ T-cell count over time post-transplant (a) after ATG treatment (b) in the comparator group receiving anti-RIL-2 ab. D, day; Y, year.

10 years, reaching a plateau after 10 years post-transplant (651 ± 287/mm^3 at 21 years) without ever regaining the pretransplant value. Interestingly, the CD4$^+$ T-cell count varied widely among patients with persistent CD4$^+$ T-cell lymphopenia (≤200/mm^3), who comprised 48.5% of patients at 1 year, 9.2% at 3 years, 6.7% at 5 years, and 2.0% at 10 years. At 21 years, no patients had a CD4$^+$ T-cell count less than 200/mm^3, but 8% had a CD4$^+$ T-cell count less than 300/mm^3.

In patients treated with an anti-RIL-2 ab, the CD4$^+$ T-cell count remained stable from the pretransplant level to 1 and 5 years post-transplantation (800 ± 365/mm^3, 770 ± 382/mm^3, and 791 ± 374/mm^3, respectively) (Fig. 2b). The CD4$^+$ T-cell was below 200/mm^3 in only 0.7% and 1.0% of these patients at 1 and 5 years, respectively.

CD8$^+$ T-cell reconstitution

Mean CD8$^+$ T-cell count increased very rapidly after the initial depletion and had recovered to pretransplantation values (463 ± 227/mm^3) by 1 year (436 ± 379/mm^3) (Fig. 3). After 1 year, mean CD8$^+$ T-cell count remained stable until 16 years post-transplantation (494 ± 291/mm^3 at 16 years).

Early T-cell reconstitution and CD4$^+$ T-cell count at 1 year

The CD4$^+$ T-cell count at 1, 3, and 6 months post-transplant in the subpopulation of patients for whom subset counts were available was assessed according to the presence or absence of CD4$^+$ T-cell lymphopenia at 12 months. At 1, 3, and 6 months, the mean (SD) CD4$^+$ T-cell count was significantly lower in patients with CD4$^+$ T-cell lymphopenia at 12 months (62 ± 70/mm^3 versus 132 ± 154/mm^3, $P = 0.002$ at 1 month; 99 ± 62 versus 203/mm^3 ± 116/mm^3, $P < 0.001$ at 3 months and 109 ± 55/mm^3 versus 258 ± 131/mm^3, $P < 0.001$ at 6 months).

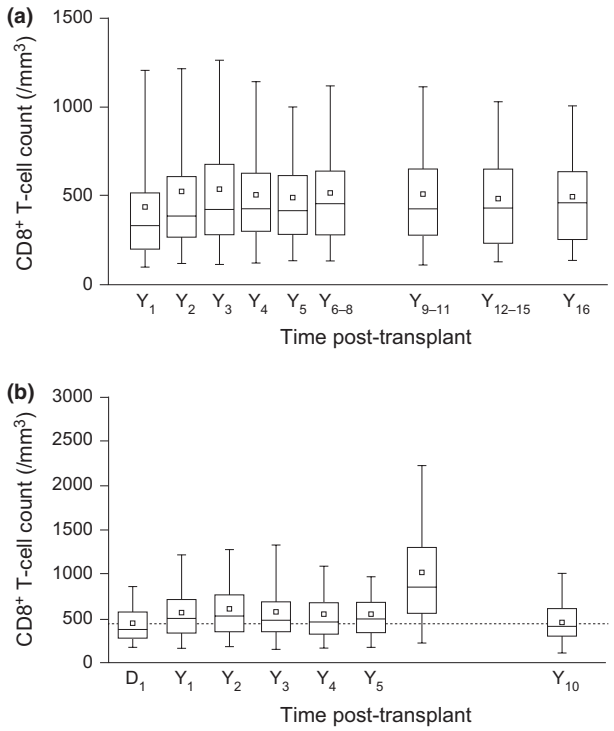

Figure 3 Box and whisker plot of CD8+ T-cell count over time post-transplant (a) after ATG treatment (b) in the comparator group receiving anti-RIL-2 ab. D, day; Y, year. Y, year.

Table 2. Univariate analysis of potential predictors for long-term CD4+ T-cell count after rATG treatment.

	Univariate analyses		
	Beta	95% CI	*P* value
Age at baseline (per year)	−0.0175	−0.0205 to −0.0144	<0.001
Female gender	0.056	0.00239 to 0.1888	0.044
Pretransplant absolute lymphocyte count (/mm^3)	0.00015	0. 00006 to 0.00025	0.002
Pretransplant CD4+ T-cell count (/mm^3)	0.0006	0.0005 to 0.0008	<0.001
CD4+ T-cell count ≤200/mm^3 at 1 year	−0.8000	−0.8766 to −0.7235	<0.001
Total dose of rATG (mg/kg)	0.0279	0.0181 to 0.0377	<0.001
Tacrolimus (reference cyclosporine)	−0.3381	−0.4334 to −0.2428	<0.001
Mycophenolate mofetil (reference azathioprine)	−0.3081	−0.3983 to −0.2179	<0.001
Acute rejection during the first year	0.1914	0.0898 to 0.2929	<0.001
Time after transplantation (per year)	0.1189	0.0827 to 0.1551	<0.001

Risk factors for long-term impaired CD4+ T-cell reconstitution

On univariate analyses, the following pretransplant characteristics were associated with long-term impaired CD4+ T-cell reconstitution: recipient age at baseline, female gender, and pretransplant absolute lymphocyte and CD4+ T-cell counts (Table 2). Post-transplant factors associated with long-term impaired CD4+ T-cell reconstitution were the total dose of rATG treatment, the CD4+ T-cell count at 12 months, immunosuppressive treatment at 1 year (tacrolimus versus cyclosporine, and MMF versus azathioprine), acute rejection during the first post-transplant year, and the time since transplantation.

On multivariate analyses, three models were analyzed. These assessed pretransplant absolute lymphocyte count, pretransplant CD4+ T-cell counts, and CD4+ T-cell count at 1 year separately because these three explanatory variables were linked and colinear. For all three models, independent predictors of long-term impaired CD4+ T-cell reconstitution were recipient age at baseline, immunosuppressive treatment at 1 year (tacrolimus versus cyclosporine, and MMF versus azathioprine) and time since transplantation (Table 3). Pretransplant CD4+ T-cell counts and CD4+ T-cell count at 1 year were associated with long-term impaired CD4+ T-cell reconstitution,

The cohort of ATG-treated patients was divided into two groups based on the period of transplantation (1986–1998 and 1998–2008). Results were very similar in both periods: age and total CD4+ T-cell count prior to transplantation were significantly associated with long-lasting lymphopenia, albeit with a shorter long-term follow-up in the more recently transplanted group. Moreover, CD4+ T-cell lymphopenia at 1 year was associated with an increased risk of long-term lymphopenia in both time periods.

Cutoff point for recipient age as a predictor of long-term CD4+ T-cell r*econstitution*

As recipient age was associated with long-term impaired CD4+ T-cell reconstitution and was a known risk factor pretransplant, possible cutoff values for this parameter were explored (Fig. 4). Recipient age greater than 40 years appeared to be significantly associated with impaired long-term CD4+ T-cell reconstitution. In the comparator group, patients aged over 40 years had a lower CD4+ T-cell count than younger patients (data not shown).

Table 3. Multivariate analyses of potential predictors for long-term CD4+ T-cell count after rATG treatment (a) model with pretransplant CD4+ T-cell count (b) model with pretransplant absolute lymphocyte count and (c) model with CD4+ T-cell ≤200/mm^3 at 1 year.

	Multivariate analyses		
	Beta	95% CI	*P* value
(a)			
Age at baseline (per year)	−0.0124	−0.0163 to −0.0085	<0.001
Female gender	0.0188	−0.0777 to 0.1152	0.703
Pretransplant CD4+ T-cell count (/mm^3)	0.0005	0.0004 to 0.0007	<0.001
Total dose of rATG (mg/kg)	0.0023	0.0158 to 0.0112	0.734
Tacrolimus (reference cyclosporine)	−0.1365	−0.2414 to −0.0317	0.011
MMF (reference azathioprine)	−0.1844	−0.2934 to −0.0754	0.001
Acute rejection during the first year	0.0542	−0.0545 to 0.1631	0.328
Time after transplantation (per year)	0.1442	0.1296 to 0.1589	<0.001
(b)			
Age at baseline (per year)	−0.0176	−0.0215 to −0.0137	<0.001
Female gender	0.0347	−0.0622 to 0.1317	0.482
Pretransplant absolute lymphocyte count (/mm^3)	0.00003	0.0005 to 0.0001	0.385
Total dose of rATG (mg/kg)	0.0016	−0.0108 to 0.0141	0.792
Tacrolimus (reference cyclosporine)	−0.1330	−0.2412 to −0.02484	0.016
MMF (reference azathioprine)	−0.2209	−0.3334 to −0.1085	<0.001
Acute rejection during the first year	0.0751	−0.0370 to 0.1873	0.189
Time after transplantation (per year)	0.1357	0.1218 to 0.1496	<0.001
(c)			
Age at baseline (per year)	−0.0096	−0.0124 to −0.0067	<0.001
Female gender	0.0550	−0.0160 to 0.1261	0.129
CD4+ T-cell < 200/mm^3 at 1 year	−0.6899	−0.7640 to −0.6159	<0.001
Total dose of rATG (mg/kg)	−0.0005	−0.0110 to 0.0099	0.919
Tacrolimus (reference cyclosporine)	−0.0937	−0.1714 to −0.0161	0.018
MMF (reference azathioprine)	−0.1369	−0.2361 to −0.0376	0.007
Acute rejection during the first year	0.0597	−0.0218 to 0.1414	0.151
Time after transplantation (per year)	0.1352	0.1217 to 0.1487	<0.001

Discussion

In this very long term up to 21 years retrospective analysis of CD4+ T-cell reconstitution following rATG treatment of kidney transplant recipients, mean lymphocyte CD4+ T-cell count increased during the first 10 years then stabilized during the following 10 years but remained below the pretransplant level even at 21 years post-transplant. Furthermore, a small proportion of patients continued to have a CD4+ T-cell count below 200/mm^3 even at 10 years (2.0%). Notably, 8.0% of patients had a CD4+ T cell, which was below 300/mm^3 after 21 years, a threshold which defines idiopathic CD4 lymphocytopenia [15]. We did not observe a significant decrease in CD4+ T-cell count between pretransplant levels and 10 years post-transplant after anti-RIL-2 ab treatment.

Risk factors for impaired CD4+ T-cell reconstitution at the time of transplant were increasing age and low pretransplant CD4+ T-cell count. At 1 year after transplantation, a low CD4+ T-cell count and treatment with MMF or tacrolimus were associated with an impaired CD4+ T-cell reconstitution.

Several studies have analyzed short-term (≥1 year) [5,6] or mid-term (up to 5 years) [8] changes in lymphocyte subsets in kidney transplant patients after rATG treatment, but no trial has previously investigated changes over the very long term. Clinicians are well aware of the short-term effects of lymphocyte-depleting antibodies on T-cell count, but the expanding use of rATG induction necessitates a better understanding of its long-term impact. Some studies have reported that CD4+ T-cell lymphopenia is associated with infectious complications [16] in cancer [17], cardiovascular complications, and mortality [8,18], but none has analyzed risk factors for impaired long-term CD4+ T-cell reconstitution in rATG-treated patients.

The current analysis suggests that there is an age-dependent decline in the capacity of the adult immune system to regenerate CD4+ T cells after rATG administration. This has already been reported in other clinical settings (HIV infection and bone marrow transplantation) [19–21] and

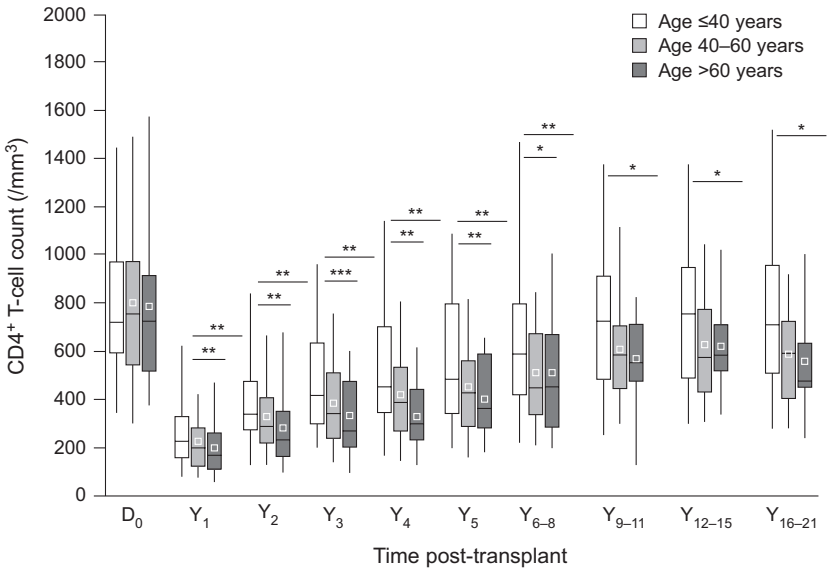

Figure 4 Box and whisker plot of CD4 + T-cell count over time post-transplant according to age ($N = 589$). D, day; Y, year. *$P < 0.050$; **$P < 0.010$; ***$P < 0.001$.

in kidney transplant patients [8,9]. One of the most striking changes in immunosenescence (the age-related decline in immune function) is involution of the thymus, which limits the production of naïve $CD4^+$ T cells [22,23]. These changes start early in life and become more pronounced after 50 years of age. Here, we identified a cutoff value of 40 years, above which impaired long-term $CD4^+$ T-cell reconstitution is significantly more likely, which may help clinicians when making immunosuppressive therapy decisions. Interestingly, $CD4^+$ T-cell count tended to be lower in older patients even in those patients who had received an anti-RIL-2 ab.

Furthermore, a low pretransplant $CD4^+$ T-cell count was a risk factor for impaired $CD4^+$ T-cell reconstitution, independent of age. It is well known that patients suffering from end-stage renal disease have impaired cellular and humoral responses [24] and a lower absolute lymphocyte count than healthy individuals [25,26]. This could be due to dysfunction of the immune system acquired prior to transplantation, perhaps due to factors related to chronic renal disease, duration of dialysis, or previous immunosuppressive treatments. Such factors occurring before transplantation may affect T-cell immune reconstitution [27].

Our findings showed that $CD4^+$ T-cell lymphopenia at 12 months or even 1 month post-transplant is associated with an impaired $CD4^+$ cell reconstitution in the long term. $CD4^+$ T-cell count at 1 month may therefore be an early indicator of impaired immune reconstitution in the future and could prompt reevaluation of the immunosuppressive regimen.

Multivariate analysis demonstrated that treatment with MMF or tacrolimus showed a significant association with reduced long-term $CD4^+$ T-cell count compared to azathioprine or cyclosporine, respectively. The influence of these drugs on T-cell reconstitution has not yet been described. Vacher-Coponat *et al.* [27] observed an impaired immune reconstitution of NK cells at 1 year after kidney transplantation using a combination of tacrolimus/MMF compared to cyclosporine/azathioprine, both with rATG induction and prednisone. They did not observe any influence on the $CD4^+$ T-cell count, but the number of patients was small.

In contrast to previous studies, we did not observe any association between the total dose of rATG and long-term $CD4^+$ T-cell lymphopenia. Of the clinical studies that have previously explored different rATG doses in kidney transplantation [28–31], only two have analyzed immune reconstitution [30,31] In a prospective study, Wong *et al.* compared immune reconstitution at 1 year between patients who received a three-day course of rATG at a dose of 1.5 mg/kg/day or 1 mg/kg/day and reported a significantly more profound and sustained depletion of $CD4^+$ T cells with the 1.5 mg/kg/day regimen [30]. Patients with a low total dose of rATG showed early $CD4^+$ T-cell count recovery at 7 days post-transplant, with complete $CD4^+$ T-cell recovery by 1 month. Kho *et al.* have recently confirmed that patients with low total dose (≤3 mg/kg) exhibit early $CD4^+$ T-cell reconstitution and only patients who received a total dose of 6 mg/kg continued to have a reduced $CD4^+$ T-cell count after 1 year [31]. In our cohort, the standard total rATG dose was 6 mg/kg,

conforming to the recommended dosing schedule of 1.5 mg/kg/day for 4 days, and few patients received a low dose (3 mg/kg).

The retrospective nature of our study necessarily limits the robustness of the conclusions. Moreover, the study period was long (1986–2009), and the rATG induction regimen changed over time with a higher cumulative dose and longer duration before 2000. However, neither the year of transplantation nor the dose or duration of rATG treatment was significantly associated with long-term impaired $CD4^+$ T-cell reconstitution on multivariate analysis. Finally, we assessed only the total $CD4^+$ T-cell count. A phenotypic analysis of memory and naïve T-cell subsets might have provided a greater understanding of immune reconstitution after rATG treatment.

In conclusion, the clinical impact of impaired immune reconstitution after rATG administration justifies pretransplant screening to identify kidney transplant patients who are at increased risk of long-term $CD4^+$ T-cell lymphopenia. Recipient age greater than 40 years, and a low $CD4^+$ T-cell count, may serve as useful predictors of risk. Moreover, a low $CD4^+$ T-cell count during the first few months post-transplant may be a marker of over-immunosuppression, suggesting that early monitoring of $CD4^+$ T cells may be helpful to guide decisions on subsequent immunosuppression in older patients and/or those with low pretransplant $CD4^+$ T-cell count.

Authorship

HL and BS: wrote the paper. HL, BS, GT, JH, YL, CB and MB: designed research/study. PG, GT, JH, YL, CB and MB: important reagents. HL, BS and GT: performed research/study. HL, CB and JM: collected data. HL, BS and JH analyzed data.

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