

Effects of Intraoperative versus Postoperative Administration of Rabbit Antithymocyte Antibodies on 1-Year Renal Function in Renal Transplant Patients

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Objectives: The aim of our study was to prospectively assess 1-year allograft outcomes and the evolution of lymphocyte subsets in a group of renal transplant patients who had received intraoperative rabbit antithymocyte antibodies (RATG).

Materials and Methods: We compared 1-year allograft transplant outcomes in renal transplant recipients who had received intraoperative RATG (group 1, n = 53) with the outcomes observed in patients in a historical control group who had received postoperative RATG (group 2, n = 49). RATG were given at the same dosage (1 mg/kg) during the first 3 days, and then the dosage was adapted according to CD2 count, until calcineurin inhibitors were started.

Results: The overall dosage of RATG administered was significantly lower in group 1. At day 4, CD2, CD3, and CD19 T-cell subset counts were significantly higher in patients in group 1. From 3 months after transplantation, CD4/CD8 ratios were significantly lower in patients in group 1 because of a rapid regeneration of CD8 T cells. One-year total lymphocyte counts were significantly higher in patients in group 1. There were fewer severe infectious complications in patients in group 1. One-year renal function was better in patients in group 2. Donor age was the only independent factor associated with renal function at both 1 month and 1 year after transplantation.

Conclusions: When RATG are infused intraoperatively, a lower total amount of RATG is required to

prevent acute rejection as compared with postoperative RATG infusion. Consequently, fewer serious lymphopenia-associated complications are observed during the first year after transplantation

Key words: Administration mode, Delayed graft function, Lymphocyte subset, Rabbit antithymocyte globulins, Renal function

The action of rabbit antithymocyte antibodies (RATG; Sangstat, Lyon, France), one of the most widely used polyclonal antibodies, is rapid, antigen-specific, and dependent on antibody dosage. Thymoglobulin contains a large number of antibodies directed against molecules involved in the immune response, that is, the T-cell receptor (TCR)/CD3 complex; CD4, CD8, and costimulatory molecules (CD 80, CD 86, CD 152...); numerous leukocyte adhesion molecules; and molecules involved in signal transduction (CD45). In addition to T-cell depletion, RATG have other mechanisms of action: RATG induce a complement-dependent lysis of dendritic cells, consequently inhibiting T-cell activation and proliferation. After binding T cells, RATG also induce an internalization of antigen-antibody complexes, leading to a down-regulation of TCR/CD3, CD4, CD8, and CD2.

It has been shown that initial delayed graft function (DGF) is associated with an increased incidence of acute rejection and a decrease in long-term renal allograft survival [1, 2, 3]. The effect of RATG upon adhesion molecules seems to be crucial in minimizing ischemia-reperfusion injury after renal transplantation, and consequently, in reducing the incidence of, and the severity of, DGF. Recently, Goggins and coworkers [4] reported that when compared with postoperative administration, intraoperative administration of RATG was associated with a reduced rate of DGF and lower serum creatinine levels at 10 and 14 days posttransplantation.

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They suggested that these better results were related to the action of RATG on ischemia/reperfusion injury. The aim of our study was to prospectively assess 1-year allograft outcomes and the evolution of lymphocyte subsets in a group of renal transplant patients who had received intraoperative RATG. These results were compared with patients in a historical control group who had received postoperative RATG at our institution before we modified the mode of RATG administration.

Materials and Methods

RATG, as induction therapy, have been used in our institution since the early 1970s and are administered immediately after transplantation. In September 2000, we modified this administration by starting the infusion 1 hour before the allograft was reperfused (ie, intraoperatively) and maintaining the infusion for 4 hours. Between September 2000 and August 2003, 300 renal transplantations were performed. Of these, 247 were not included in this study because they had received either induction therapy with anti-CD25 monoclonal antibodies or sirolimus-based therapy, according to clinical trial parameters. Thus, 53 patients received intraoperative RATG (group 1) followed by triple immunosuppressive therapy containing a calcineurin inhibitor and were included in this study.

The efficacy and safety (see below) of intraoperative RATG administration were compared with those observed in a historical control group (group 2). Between January 1998 and September 2000, 192 patients underwent renal transplantation in our department; of these, 143 received anti-CD 25 or no induction therapy. The 49 remaining patients were treated with postoperative RATG and were included in this study. All patients were white and received a sequential quadruple immunosuppressive therapy based on a calcineurin inhibitor, that is, cyclosporine A (Novartis Pharma, Basel, Switzerland) or tacrolimus (Fujisawa, München, Germany) in addition to azathioprine or mycophenolate mofetil (MMF) (Roche, Basel, Switzerland) and corticosteroids. Prior to September 2000, the first infusion of RATG (1 mg/kg) was given after completing the transplantation over a 6-hour period (day 1). After September 2000, it was given during transplantation, beginning 1 hour before completion of the vascular anastomosis. Thereafter, in both groups, infusion was repeated at the same dosage on days 2 and 3, and then the dosage of each

infusion was adapted to maintain a CD2 count below 50/mm³. RATG were administered until calcineurin inhibitors were started. At 30 minutes before RATG infusion, patients were administered 500 mg paracetamol IV, 50 mg diphenhydramine, and 1 mg/kg of methylprednisolone, except on the first 2 days (see below). In addition, pulse methylprednisolone (10 mg/kg methylprednisolone) was given 1 hour preoperatively. Calcineurin inhibitors were begun when the serum creatinine level had fallen below 220 μ mol/L. Cyclosporine A was started at a dosage of 6 mg/kg/day, and then the dosage was adapted until a cyclosporine A trough level between 150 and 250 ng/mL during the first year could be achieved. Tacrolimus was started at a dosage of 0.1 mg/kg/day, and tacrolimus trough levels were maintained between 10 and 15 ng/mL. Before transplantation, each patient received 100 mg of azathioprine IV or 2 g of MMF. With regard to prednisolone, patients received 250 mg IV on day 1, 125 mg IV on the second day, and then 1 mg/kg/day orally for 7 days. The dosage was then tapered to 10 mg/day at 3 months and finally, to 5 mg/day thereafter. A posttransplant increase in serum creatinine level was always investigated by a kidney biopsy after ruling out urine obstruction or overt calcineurin overdosage. Acute cellular rejection (defined by Banff classification) was treated with steroid pulse therapy (10 mg/kg) for 3 days. In cases of steroid-resistant rejection, patients received either antithymocyte globulins (1 mg/kg) for 6 days or OKT3 (5 mg/day) for 10 days.

In patients at high risk for cytomegalovirus (CMV) (ie, a seropositive donor donating to a seronegative recipient), a systematic sequential prophylaxis using ganciclovir (10 mg/kg/day IV adapted to renal function) was administered for the first 2 weeks after transplantation followed by oral valganciclovir (3 g/day adapted to renal function) for the following 3 months. In the other patients, close monitoring of CMV viremia by real-time polymerase chain reaction was performed. With respect to CMV infection, reactivation was defined on the basis of viremia during the first 6 months after transplantation. Symptomatic CMV disease was defined according to agreed criteria [5] and included either a fever greater than 38°C for 48 hours in the absence of bacterial or fungal infection or rejection, and/or a progressive falling neutrophil count for 3 days, and/or thrombocytopenia at $< 100 \times 10^9/L$, or involvement of an organ. In cases of CMV reactivation or disease, patients were treated with ganciclovir (10 mg/kg/day IV

adapted to renal function) for 14 to 21 days. All patients received anti-*Pneumocystis carinii* prophylaxis during the first 6 months after transplantation using 400 mg sulfamethoxazole-trimethoprim every other day.

Results are presented as means $\pm$ SE except when the spread of the values is non-Gaussian: in those cases, we used the median (ranges). Quantitative parameters were compared using unpaired two-sample *t* tests or the nonparametric Mann-Whitney *U* test. Qualitative parameters were compared using Fisher's exact test. Independent factors associated with a 1-month serum creatinine level below 120 μ mol/L and a 1-year serum creatinine level below 100 μ mol/L were studied using a stepwise multivariate logistic regression model. A value for *P* below .05 was considered significant.

Results

Demographic data are summarized in Table 1. In both groups, recipients' demographic data and the transplantation characteristics were comparable, while donors' characteristics were different. Donors were

significantly older in group 1 than they were in group 2 (*P* = .002). Significantly more donors in group 1 died from vascular disease (*P* = .02). In contrast, donors' serum creatinine levels and creatinine clearance levels were similar in both groups.

During RATG administration, the CD2 count was maintained within the target range (ie, $< 50/\text{mm}^3$, see below). However, the overall dosage of RATG administered as an induction therapy was significantly lower in patients in group 1 compared with patients in group 2 (5.74 ± 0.28 vs 6.7 ± 0.29 mg/kg, respectively; *P* = .02). Although it did not reach a level of significance, the number of days of treatment by RATG was lower in group 1 than it was in group 2 (5.03 ± 0.34 vs 5.83 ± 0.25 ; *P* = .06). The number of patients receiving cyclosporine A or tacrolimus was similar in the 2 groups (Table 2). In contrast, the number of patients receiving MMF was significantly higher in group 1 (98% vs 43%, *P* < .0001). The daily dosages of cyclosporine A, tacrolimus, MMF, azathioprine, and steroids, as well as cyclosporine A and tacrolimus trough levels during the first, third, sixth, and 12th months after transplantation were similar in the 2 groups (data not shown).

Table 1. Demographic data and renal transplantation characteristics

	Group 1 (intraoperative RATG) (n = 53)	Group 2 (postoperative RATG) (n = 49)	P value
Recipients (R)			
Age (y)	46 $\pm$ 2	45 $\pm$ 2	ns
Gender (M/F)	35/18	28/21	ns
Initial nephropathy			ns
Glomerulonephritis	23	20	ns
Interstitial nephropathy	12	8	
Vascular nephropathy	3	5	
Polycystic disease	7	10	
Other	8	6	
Time on dialysis (mo)	69 $\pm$ 8	59 $\pm$ 9	ns
Hemodialysis/peritoneal dialysis	52/1	46/3	ns
Number of previous RT	0 (range, 0-2)	0 (range, 0-2)	ns
EBV seropositive	51	47	ns
HCV seropositive	4	6	ns
PRA max (%)	0 (range, 0-100)	0 (range, 0-100)	ns
PRA at transplantation (%)	0 (range, 0-90)	0 (range, 0-54)	ns
Donors (D)			
Age (y)	45 $\pm$ 2	36 $\pm$ 2	.002
Cadaveric/living donor	51/2	46/3	ns
Death related to cardiovascular disease (%)	53	29	.02
Serum creatinine level (μ mol/L)	111 $\pm$ 11	95 $\pm$ 5	ns
Creatinine clearance (mL/min)	94 $\pm$ 6	104 $\pm$ 5	ns
EBV seropositive	47	37	ns
Cold ischemia time (hours)	21.7 $\pm$ 1.2	22.4 $\pm$ 1.2	ns
Warm ischemia time (hours)	0.9 $\pm$ 0.05	0.8 $\pm$ 0.04	ns
HLA A/B/DR matching	3 (range, 0-6)	3 (range, 0-6)	ns
HLA DR matching	1 (range, 0-2)	1 (range, 0-2)	ns
CMV status			
R+	31	27	ns
D+/R-	12	7	ns
D-/R-	10	15	ns

RATG, rabbit antithymocyte globulins; M, male; F, female; RT, renal transplantation; EBV, Epstein-Barr virus; HCV, hepatitis C virus; PRA, panel reactive antibodies, HLA, human leukocyte antigen; CMV, cytomegalovirus, R, recipient; D, donor

Impact on renal allograft

Delayed graft function: The number of DGFs, defined as the need for at least 1 hemodialysis session after renal transplantation, was similar in both groups (16 of 53 [30%] in group 1, and 14 of 49 [28.5%] in group 2, $P > .05$). In contrast, when hemodialysis was required, the number of hemodialysis sessions was higher in the first group, even if this result was not significant (ie, 3 [1-16] in group 1 vs 2 [1-3] in group 2; $P = .06$; Table 3). The mean length of stay after transplantation was similar in both groups (15 days [range, 8-86 days] in group 1 and 16 days [range, 8-33 days] in group 2). The time to reach a serum creatinine level below $220 \mu\text{mol/L}$ was similar in both groups (ie, 12 ± 2 vs 8 ± 1 days; $P = .09$). In 2 patients from the first group, serum creatinine levels after transplantation remained above $220 \mu\text{mol/L}$.

Renal function: Serum creatinine level and creatinine clearance were similar in both groups at days 7, 15, and 30. At the third month after transplantation and thereafter, serum creatinine levels remained significantly higher in group 1 compared with group 2 (Figure 1). In contrast, the nadir of serum creatinine levels during the first and third months after transplantation was significantly lower in group 2 (131 ± 7

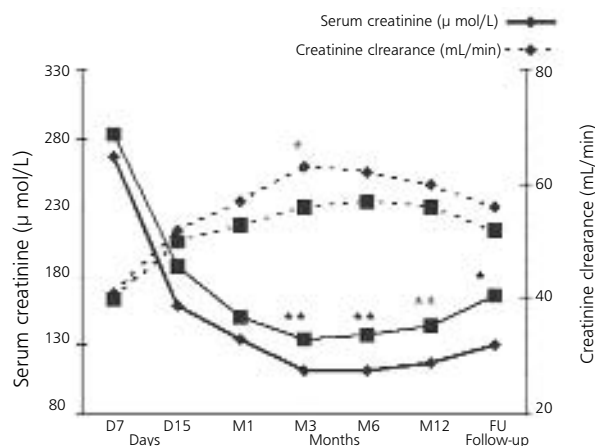


Figure 1. Patterns of posttransplant mean values of serum creatinine levels and creatinine clearances in renal transplant patients who had received induction therapy with either intraoperative (- ■ -) or postoperative (- ♦ -) RATG.

* $P < .05$; ** $P < .01$

$\mu\text{mol/L}$ in group 1 vs $107 \pm 5 \mu\text{mol/L}$ in group 2 during the first month [$P = .006$] and $118 \pm 5 \mu\text{mol/L}$ in group 1 vs $96 \pm 4 \mu\text{mol/L}$ in group 2 during the 3 first months [$P = .002$]). There were no differences in serum creatinine levels and creatinine clearances at the third, sixth, and 12th months after transplantation between patients with or without DGF (data not shown). Proteinuria was similar in both groups at 3, 6, and 12 months after transplantation (data not shown).

Table 2. Immunosuppressive treatment

	Group 1 (intraoperative RATG) (n = 53)	Group 2 (postoperative RATG) (n = 49)	P value
Total RATG dosage delivered (mg)	370 ± 16	412 ± 20	ns
RATG administrated per dose (mg/kg)	5.7 ± 0.28	6.7 ± 0.29	.02
Number of RATG treatment (days)	5 ± 0.34	5.83 ± 0.25	ns
Duration of initial hospitalization (days)	21 ± 2	18 ± 1	ns
Cyclosporine A/tacrolimus	38/14	33/13	ns
MMF/azathioprine	52/1	21/28	< .0001

RATG, rabbit antithymocyte globulins; MMF, mycophenolate mofetil

Table 3. Efficacy and safety of intraoperative vs postoperative administration of RATG in renal transplant patients

	Group 1 (intraoperative RATG) (n = 53)	Group 2 (postoperative RATG) (n = 49)	P value
Delayed graft function (%)	30	28.5	ns
Number of hemodialysis sessions needed	3 (range, 1-16)	2 (range, 1-3)	ns
Acute rejection (AR) (%)	20.75	18.36	ns
Steroid-resistant AR	1	2	ns
Time between renal transplantation and AR (d)	77 ± 21	32 ± 10	ns
Fever-related to RATG (%)	17	37	.04
CMV infection (%)	50	40	ns
Serious infections requiring hospitalization*	6	16	.01
Urinary tract infection	4	4	
Pneumonia	2	3	
Gastrointestinal infection	0	3	
Herpes simplex infection	0	3	
Other†	0	3	

*excluding CMV infections

†1 peritonitis, 1 osteitis, and 1 *Varicella zona* infection

RATG, rabbit antithymocyte globulins; AR, acute rejection; CMV, cytomegalovirus

The number of patients with hypertension, the number of patients receiving ACE inhibitors and/or AT-1 receptor blockers, and the number of patients receiving HMG-coA inhibitors were similar in both groups (data not shown).

Acute rejection: Overall, 20 patients presented with an acute rejection: 11 from group 1 and 9 from group 2; this result was not significant. Of these, 3 were steroid-resistant: 1 patient from group 1 was treated with antithymocyte globulins, and 2 patients from group 2 were treated with OKT3. The number of acute rejections in each group and the time between renal transplantation and the first acute rejection were similar in both groups (Table 3).

Effects on hematologic parameters and lymphocyte subsets

Before renal transplantation, hemoglobin level, leukocyte and platelet counts, total lymphocyte counts, and lymphocyte subset counts were similar in both groups. After transplantation, there was a significant decrease in all previous hematologic parameters. However, at day 30, hemoglobin levels were higher in

group 1 compared with group 2 ($P = .0002$). Leukocyte count was always greater in group 1 than in group 2, although the difference was only significant at day 15 and at 1 year after transplantation (respectively, $P = .05$ and $P = .04$). Conversely, platelet counts were always higher in group 2 than they were in group 1, although the difference was only significant at day 15 ($P = .01$). Finally, at days 7 and 15, and after 1 year, total lymphocyte counts were significantly higher in group 1 than they were in group 2 ($P = .0007$, $P = .02$, and $P = .004$) (Figure 2). With respect to CD2 lymphocyte counts during RATG administration, the total number of CD2 counts was always below the target ($< 50/\text{mm}^3$). However, at day 4, after a similar dosage of RATG had been administered to patients in both groups (ie, 1 mg/kg/day for 3 consecutive days), CD2, CD3, and CD19 T-cell counts were significantly higher for those in group 1 compared with those in group 2 (35 ± 5 vs $10 \pm 2/\text{mm}^3$, $P < .0001$ for CD2 T cells; 25 ± 4 vs $6 \pm 2/\text{mm}^3$, $P = .0003$ for CD3 T cells; and 179 ± 22 vs $76 \pm 14/\text{mm}^3$, $P = .0004$ for CD19 T cells). At day 7, CD2, CD3, and CD19 T-cell counts were also significantly higher in group 1 than they were in group 2 (55 ± 13 vs $19 \pm 6/\text{mm}^3$, $P < .02$ for

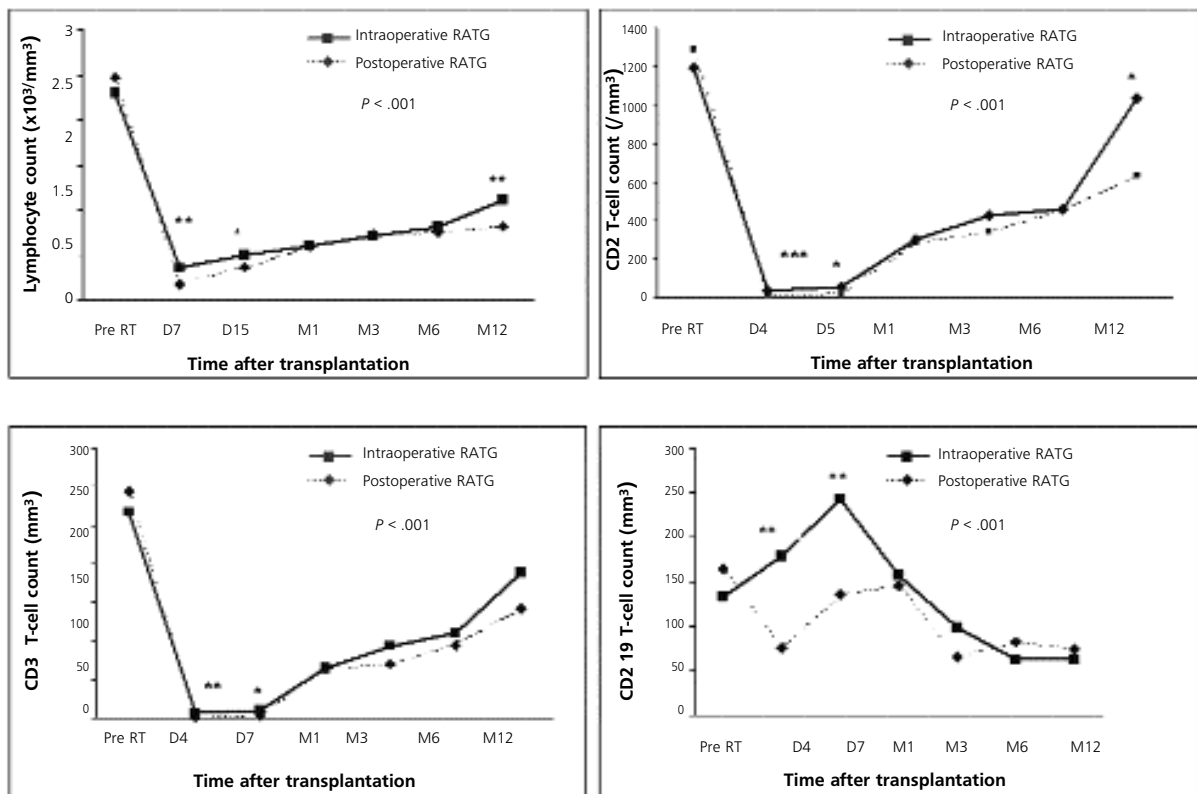


Figure 2. Patterns of mean, total lymphocytes, CD2, CD3, and CD19 T-cell subsets during the 1st year after transplantation in renal transplant patients receiving induction therapy with either intraoperative (—■—) or postoperative (---♦---) RATG.

Pre RT, before renal transplantation; D, day; M, month

* $P < .05$; ** $P < .01$; *** $P < .0001$

CD2 T cells; 43 ± 12 vs $14 \pm 6/\text{mm}^3$, $P = .050$ for CD3 T cells; and 244 ± 29 vs $136 \pm 20/\text{mm}^3$; $P = .003$ for CD 19 T cells). Thereafter, CD2, CD3, and CD 19 T-cell counts became similar in the 2 groups, except that the CD2 T-cell count was significantly higher in group 1 than it was in group 2 at 12 months after transplantation (1072 ± 241 vs $638 \pm 65/\text{mm}^3$, $P = .04$; Figure 2). The evolution of CD4 and CD8 T-cell counts was similar in both groups during follow-up, except that CD8 T-cell counts were significantly higher in group 1 than they were in group 2 at 12 months posttransplantation (516 ± 76 vs $327.5 \pm 40/\text{mm}^3$, $P = .02$). CD4/CD8 ratios were significantly higher in group 2 than they were in group 1 at 3, 6, and 12 months after transplantation (Figure 3). Finally, the evolution of natural killer cells was similar in both groups (Figure 3).

Patient and renal allograft survival

One-year patient survival rates were 100%. Five patients underwent hemodialysis during the first year: 4 from group 1 and 1 from group 2; this result was not significant. One of the patients presented

with a suppurative lymphocele that was resistant to medical treatment. Consequently, the patient underwent an allograft nephrectomy despite a functional graft. Three patients presented with end-stage renal disease that was related to chronic allograft dysfunction. The fifth patient presented with an irreversible relapse of his initial nephropathy (ie, thrombotic microangiopathy).

Safety

ATG-specific complications: The number of fever episodes in the absence of infection (which possibly was related to RATG administration) was significantly higher in group 2 than it was in group 1 (ie, 17% vs 37%, $P = .04$). However, we observed only 1 serum sickness disease in group 1.

Infectious disease: The number of CMV infections was similar in both groups (Table 3). Five patients from group 1 (out of the 12 D+/R-, 42%) and 3 patients (out of 7 D+/R-, 43%) from group 2 developed a CMV primo-infection (not significant). A CMV reactivation was observed, respectively, in 45% and

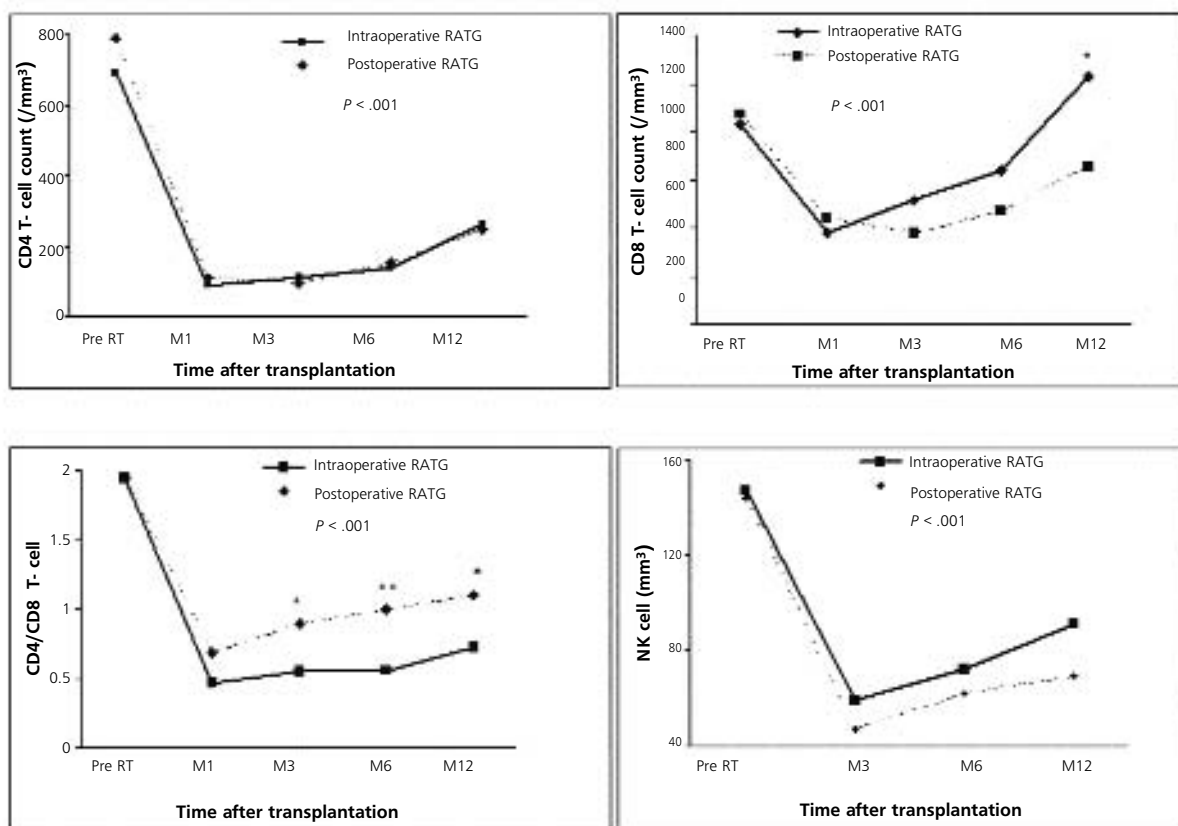


Figure 3. Patterns of mean CD4 and CD8 T-cell subsets, CD4/CD8 T-cell ratios, as well as natural killer (NK) cell subsets during the 1st year after transplantation in renal transplant patients receiving induction therapy with either intraoperative (-■-) or postoperative (-◆-) RATG. Pre RT, before renal transplantation; D, day; M, month
* $P < .05$; ** $P < .01$. *** $P < .0001$

41% of the CMV seropositive recipients from groups 1 and 2 (not significant). Three patients presented with CMV disease: 1 from group 1 and 2 from group 2 (not significant). In contrast, the number of serious infections (except CMV) that required hospitalization was higher in group 2: 11% from group 1 vs 33% from group 2 ($P = .01$).

Independent factors associated with serum creatinine level at 1 month and 1 year after renal transplantation

Using a univariate analysis, we identified factors at 1 month posttransplantation that were associated with serum creatinine levels below 120 $\mu\text{mol/L}$ (ie, the median value) and factors at 1 year posttransplantation that were associated with normal serum creatinine levels (ie, $\leq 100 \mu\text{mol/L}$). The independ-

ent factors associated with serum creatinine outcomes were studied using a multivariate logistic regression model. For the analysis at 1 month, we only included the mode of RATG administration in the model and the variables for which the P value was $\leq .05$ in the univariate analysis (Table 4). Donor age below 40 years and serum creatinine level at day 7 were significantly associated with a 1-month serum creatinine level below 120 $\mu\text{mol/L}$ ($P = .03$ and $P = .02$, respectively). At 1 year, we included the mode of RATG administration in the model and all parameters for which the P value was ≤ 0.1 in the univariate analysis (Table 5). Donor age below 40 years and serum creatinine level at 6 months were significantly associated with a 1-year serum creatinine level below 100 $\mu\text{mol/L}$ (respectively, $P = .05$ and $P = .0002$).

Table 4. Univariate and multivariate analyses (logistic regression analysis) associated with 1-month serum creatinine levels below 120 $\mu\text{mol/L}$

Univariate analysis			
Variables	SCr level $\leq 120 \mu\text{mol/L}$	SCr level $> 120 \mu\text{mol/L}$	P
Intraoperative/postoperative RATG administration mode	20/27	32/22	ns
Donor's age (y)	35 $\pm$ 2	47 $\pm$ 2	< .0001
Delayed graft function* (yes/no)	8/39	21/33	.02
Total delivered dose of RATG (mg)	347 $\pm$ 17	426 $\pm$ 18	.002
Serum creatinine level at day 7 ($\mu\text{mol/L}$)	171 $\pm$ 23	288 $\pm$ 32	.003
Multivariate logistic analysis model			
Variables	Odds ratio	95% CI	P
Intraoperative/postoperative RATG administration mode	-	-	ns
Donor's age (≤ 40 y and/or > 40 y)	2.8	1.1-7	.03
Delayed graft function* (yes/no)	-	-	ns
Total delivered dose of RATG (mg)	-	-	ns
Serum creatinine level at day 7 ($\mu\text{mol/L}$)	1.004	1.0007-1.007	.02

SCr, serum creatinine; RATG, rabbit antithymocyte globulins; ns, not significant

*Delayed graft function was defined as the need for at least 1 hemodialysis session after transplantation.

Table 5. Univariate and multivariate analyses (logistic regression analysis) associated with 1-year serum creatinine levels below 100 $\mu\text{mol/L}$

Univariate analysis			
Variables	SCr level $\leq 100 \mu\text{mol/L}$	SCr level $> 100 \mu\text{mol/L}$	P
Intraoperative/postoperative RATG administration mode	7/16	42/32	.05
Donor's age (y)	30 $\pm$ 2.5	44 $\pm$ 2	.0001
Donor's serum creatinine level ($\mu\text{mol/L}$)	84 $\pm$ 5	98 $\pm$ 4	.09
Donor's creatinine clearance level* (mL/min)	120 $\pm$ 7	94 $\pm$ 4	.002
Nadir serum creatinine level during first month ($\mu\text{mol/L}$)	83 $\pm$ 5	126 $\pm$ 5	< .0001
Serum creatinine level at 3 mo ($\mu\text{mol/L}$)	93 $\pm$ 5	130 $\pm$ 4	< .0001
Serum creatinine level at 6 mo ($\mu\text{mol/L}$)	87 $\pm$ 4	134 $\pm$ 4	< .0001
Multivariate logistic analysis model			
Variables	Odds ratio	95% CI	P
Intraoperative/postoperative RATG administration mode	-	-	ns
Donor's age (≤ 40 y and/or > 40 y)	4.6	1.1-21	.05
Donor's serum creatinine level (≤ 80 and/or $> 80 \mu\text{mol/L}$)	-	-	ns
Donor's creatinine clearance* (≤ 100 and/or $> 100 \text{ mL/min}$)	-	-	ns
Nadir serum creatinine level during the 1st month ($\mu\text{mol/L}$)	-	-	ns
Serum creatinine level at 3 mo ($\mu\text{mol/L}$)	-	-	ns
Serum creatinine level at 6 mo ($\mu\text{mol/L}$)	1.1	1.05-1.16	.0002

SCr, serum creatinine; RATG, rabbit antithymocyte globulins; ns, not statistically significant

*The creatinine clearance level was estimated by the Cockcroft and Gault formula

Discussion

Our results demonstrate that when infused intraoperatively, a lower amount of RATG was needed than when they are delivered after transplantation. Therefore, less deep lymphopenia is seen at 12 months, and consequently, fewer serious infections requiring hospitalization were observed in the intraoperative group, as immunosuppression was sufficient to avoid acute rejection.

Several mechanisms of T-cell depletion induced by RATG have been demonstrated. Opsonization, with subsequent phagocytosis by liver, spleen, and lung macrophages, contributes to eliminating RATG-coated T cells [6]. Complement-dependent lysis does not discriminate between resting and preactivated T cells [7]. Conversely, antibody-dependent cell-mediated cytotoxicity and activation-induced cell death, mediated by Fas/L-Fas interaction, target selectively activated but not resting T cells [7]. Only preactivated T cells that have received an interleukin-2 signal are susceptible to this apoptotic pathway [8]. This may explain why, in patients who had received postoperative RATG, greater circulating T-cell depletion was observed at day 4, despite similar dosages of RATG being administered in both groups. In the postoperative group, T cells were stimulated by alloantigens before receiving RATG, and hence, all the mechanisms mentioned above contribute to T-cell depletion. In addition, patients receiving postoperative RATG received an additional dose of corticosteroids before the first RATG infusion, which might have increased T-cell depletion via the apoptosis pathway. Finally, the action of antithymocyte globulins is dependent on surface antigen densities [9]. Expression of antigens might be increased on the surface of already activated T cells of patients who received RATG after renal allograft reperfusion. In addition to T-cell depletion, RATG treatment induces functional alterations. This was demonstrated by the impaired proliferative responses of lymph node cells in mixed leukocyte reactions, and down-modulation of T-cell surface signaling molecules such as CD2, CD3, CD4, and CD8 [6]. This may induce a decreased proliferative response of initially nonactivated T cells in patients receiving intraoperative RATG infusion, which results in a lower total amount of RATG being delivered to maintain a CD2 count below 50/mm³. In Goggins and coworkers' study, the total amount of delivered RATG was similar in both groups of patients [4]. The difference observed between the

present study and the one by Goggins, regarding total number of RATG doses delivered, might be related to the infusion of a fixed RATG dosage in the Goggins study, whereas we adapted the RATG dosage delivered according to the circulating T-cell subset counts.

Rabbit polyclonal agents, and not equine antilymphocytic agents, have been shown to result in long-term lymphocyte depletion and inversion of the CD4/CD8 ratio, which may persist for years [10, 11]. Müller and coworkers found that during the first 24 months after transplantation, depression of the CD4/CD8 ratio is primarily due to depletion of CD4 T-cell numbers. After regeneration of the CD4 T-cell number to near normal levels, which is dependent on the thymus, high CD8 T-cell numbers result in a persistent low CD4/CD8 ratio. Reconstitution of CD4 T cells depends on the thymus, whereas CD8 T cells can be restored by extrathymic maturation [9]. In the present study, CD8 T-cell reconstitution started as soon as 3 months after transplantation in patients who received intraoperative RATG. This was not driven by CMV infection, which is a potent cause of CD8 expansion, because the rate of CMV infection was similar in both groups. The more profound lymphopenia resulting from rabbit polyclonal agent administration might result in more infectious complications. Hence, in our study, there were significantly fewer serious infections requiring hospitalizations in the intraoperative group (in which lymphopenia was less profound) compared with the postoperative group. Finally, despite the lower total amount of RATG delivered, its intraoperative administration results in sufficient immunosuppression to avoid acute rejection.

DGF has been associated with an increase in the renal tubule surfaces of various adhesion molecules and major histocompatibility antigens [12, 13]. Recently, many authors have suggested the use of RATG induction therapy prior to allograft reperfusion to reduce leukocyte-endothelium interactions, which are enhanced by ischemia-reperfusion injury [4, 9, 14]. Michallet and coworkers showed that RATG induced a dose-dependent down-modulation of cell-surface expression of b-integrin and intercellular adhesion molecules on lymphocytes, monocytes, and neutrophils [15]. Of these, it is the leukocyte function-associated antigen-1 (LFA-1) that plays an important role in the adhesion of leukocytes to the endothelium. This step is usually followed by leukocyte extravasation and by migration to the sites of inflammation. Hence, it seems logical that the inhibition of LFA-1 by

the antibodies present in RATG might result in a decrease of leukocyte rolling, as well as their adhesion to the endothelium. This was emphasized by a video demonstration by Hammer and Thein [14], who, in a monkey model, showed that ischemia/reperfusion result in an increase in leukocyte adherence, whereas RATG almost completely inhibit the adhesion phenomenon, and rolling of leukocytes occurs less frequently. Because LFA-1 bears many epitopes, anti-LFA1 monoclonal antibodies were unable to induce in vitro LFA-1 modulation unless cross-linked by another antibody [15]. Therefore, Michallet and colleagues concluded that RATG are much more potent anti-LFA1 agents than are CD11a monoclonal antibodies.

However, in a randomized clinical trial in adult renal-transplantation patients, in which LFA-1 monoclonal antibodies and RATG as induction therapies were compared, the rate of DGF, defined as the need for at least 1 hemodialysis session during the first week, was higher in the RATG group, even if it did not reach statistical significance (19% in the LFA-1 group vs 35% in the RATG group, $P = .08$). In contrast to Goggins and coworkers' findings [4], we found no difference regarding DGF and posttransplant dialysis rate in patients receiving either intraoperative or postoperative RATG induction therapy. This might be the consequence of using less adequate renal transplants (ie, older donors) and higher cold ischemia times. After the first infusion of RATG or OKT3, tumor necrosis factor- α (TNF- α) is released into the systemic circulation; its serum level reaches a peak 1 hour later and then decreases [16, 17]. In the Goggins and coworkers' study, it was not specified by how many times or by how long before renal allograft reperfusion the RATG were infused [4]. In our study, we infused RATG for only 1 hour before the renal allograft was reperfused (ie, at the moment of the highest serum TNF- α level). This TNF- α level was found to be significantly higher in renal transplant patients experiencing DGF compared with those with immediate graft function [18]. Hence, even if our patients received a methylprednisolone pulse before RATG infusion (which might have decreased the release of TNF- α [17]), the timing of RATG infusion with respect to the allograft reperfusion also might explain the difference observed between Goggins and colleagues' study and ours. Similarly, in a large cohort of renal allograft recipients treated with antithymocyte globulins as an induction therapy, no difference was found regarding DGF between patients receiving RATG before or after allograft reperfusion [19].

Despite the better renal allograft function observed during the first month in patients who received intraoperative RATG, Goggins and coworkers reported a similar serum creatinine level at 6 months, posttransplantation in patients who received intraoperative or postoperative RATG. In the present study, as shown in Figure 1, renal function was better in patients receiving postoperative RATG. However, this was not related to the mode of administration of RATG. The worse renal function observed in the group of patients receiving intraoperative RATG is related to the modification of demographic renal donor characteristics, that is, older patients and more death related to cardiovascular diseases. During the last few years, a modification in the donors' characteristics has been observed. As shown by multivariate analyses, donor age is an independent factor associated with renal function at 1 month and at 1 year after transplantation.

In conclusion, in this nonrandomized study based on historical controls, we found that intraoperative infusion of RATG is associated with a lower overall amount of delivered RATG, and therefore, less serious lymphopenia at 12 months, posttransplantation. Consequently, infectious complications were less frequent, and immunosuppression was sufficient to avoid acute rejection. Finally, it seems better to infuse RATG before the renal allograft reperfusion because the DGF rate was similar in both groups, despite the use of more marginal kidneys in the group of patients receiving intraoperative RATG.

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