Polyclonal Antithymocyte Globulin and Cardiovascular Disease in Kidney Transplant Recipients

Didier Ducloux,*†‡ Cécile Courivaud,*†‡ Jamal Bamoulid,*†‡ Thomas Crepin,*†‡ Jean-Marc Chalopin,*†‡§ Pierre Tiberghien,*†|§ and Philippe Saas*†§

*Integrated Center for Research in Inflammatory Diseases (UMR1098), French Institute of Health and Medical Research, University Hospital Federation, Besançon, France; †French Federal Research Institute for Engineering for Cellular and Tissue Biology (IFR133), University of Franche-Comté, Besançon, France; ‡Department of Nephrology, Dialysis, and Renal Transplantation, University Hospital of Besançon, Besançon, France; §Clinical Investigation Centre–Integrated Biotherapeutics (CIC-BT 506), University Hospital of Besançon, Besançon, France; and |Biomonitoring Platform, French Blood Service Bourgogne Franche-Comté, Clinical Investigation Centre–Integrated Biotherapeutics (CIC-BT 506), Besançon, France

ABSTRACT

T-lymphocyte activation may contribute to atherosclerosis, the prevalence of which is increased in transplant patients. However, the cardiovascular consequences of polyclonal antithymocyte globulin (ATG)–induced immune modifications, which include alterations in T-cell subsets, are unknown. We conducted a retrospective single-center study to assess whether ATG associates with an increased incidence of atherosclerotic events (CVEs) in kidney transplant patients. Propensity score analysis was performed to address potential confounding by indication. We also tested whether ATG use induces a proatherogenic immune status. Sixty-nine (12.2%) CVEs occurred during follow-up (87 ± 31 months). The cumulative incidence of CVEs was higher in ATG-treated patients (14.7% versus 8.2%; P = 0.03). Cox regression analysis revealed that ATG use was an independent risk factor for CVEs (hazard ratio [HR], 2.36; 95% confidence interval [95% CI], 1.35 to 4.13; P = 0.003). Results obtained in the propensity score match analysis recapitulated those obtained from the overall cohort (HR, 2.09; 95% CI, 1.11 to 3.98; P = 0.02). Late-stage differentiated CD8+ T cells increased 1 year after transplantation only in ATG-treated patients. More generally, ATG associated with features of immune activation. These modifications increased markedly in patients exposed to cytomegalovirus (CMV). Subanalyses suggest that the effect of ATG on CVEs is restricted to CMV-exposed patients. However, CMV infection associated significantly with CVEs only in ATG-treated patients (HR, 2.07; 95% CI, 1.16 to 3.70; P = 0.01). In conclusion, ATG associated with both immune activation and post-transplant CVEs in this cohort. Further studies should precisely determine whether ATG-induced immune activation is the causal link between ATG and CVEs.


Received June 26, 2013. Accepted November 21, 2013. Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Didier Ducloux, Department of Nephrology, Dialysis, and Renal Transplantation, University Hospital of Besançon, F-25030 Besançon, France. Email: dducloux@chu-besancon.fr

Copyright © 2014 by the American Society of Nephrology

Broad T-cell depletion by polyclonal antithymocyte globulins (ATGs) has been used for many years as a part of immunosuppressive treatment in transplantation. ATGs have potent immunosuppressive properties and have been proven to be superior to anti-CD25 mAb in immunologic high-risk patients.1,2 These polyclonal antibodies are a complex mixture of antibodies with multiple specificities directed against both T and non–T cells.1,2 They produce profound T-cell depletion1,2 and induce persistent changes in T-cell subsets characterized by a low CD4+ T-cell count and CD8+ T-cell expansion.3–5 The clinical consequences of ATG-induced CD8+ T-cell depletion and CD4+ T-cell expansion.

1349
T-cell activation are poorly known. However, recent data emphasize the effect of T-lymphocyte activation in atherosclerosis. Indeed, expansion of activated CD8\(^+\) lymphocytes has been associated with cardiovascular disease (CVD) in both patients with HIV and the general population. Interestingly, an increased incidence of cardiovascular mortality has been reported in ATG-treated renal transplant recipients. Nevertheless, this registry-based study did not take into account a selection bias and results remain highly questionable. We also reported an increased incidence of cardiovascular events in patients with ATG-induced persistent CD4 T-cell lymphopenia. Unfortunately, all of the patients had received ATG and the lack of a control group did not enable to demonstrate a direct effect of ATG on atherosclerosis.

We first conducted a retrospective single-center study to assess whether ATG is associated with an increased incidence of atherosclerotic events. Propensity score analysis was performed to address potential confounding by indication. We also tested whether ATG may induce an expansion of activated CD8\(^+\) T cells in transplant patients.

**RESULTS**

**Study Population**
Baseline characteristics according to the exposure groups are shown in Table 1. The patients were followed for a mean duration of 87 ± 31 months.

**Determinants of ATG Use**
Logistic regression revealed that age (P<0.001), presence of anti-HLA antibodies (P=0.02), previous kidney transplantation (P=0.001), number of HLA mismatches (P=0.03), a previous history of CVD (P=0.04), hypercholesterolemia (P=0.003), donor age (P=0.01), and study period (P<0.001) were associated with ATG use.

On the basis of this model, the propensity score was assessed for each patient (mean 0.61 ± 0.21; range, 0.17–0.98). The area under the curve of the propensity score was 0.75 (range, 0.71–0.78).

Propensity score matching successfully balanced the distribution of characteristics across patients who were treated with ATG compared with those who were not (Table 2). Mean propensity scores were similar in patients who experienced acute rejection compared with those who did not.

**Death-Censored CVEs**
Sixty-nine CVEs (12.2%) occurred during follow-up. This corresponds to 18 CVEs for 1000 patients per year. Mean follow-up was higher in ATG-treated patients (101 ± 42 versus 87 ± 40 months; P=0.01). The cumulative incidence of CVEs was higher in ATG-treated patients (14.7% versus 8.2%; P=0.03) (Figure 1A).

In univariate analysis, age (P=0.004), a past history of CVD (P=0.004), pretransplant diabetes (P=0.06), hypercholesterolemia (P=0.04), cytomegalovirus (CMV) infection (P=0.03), smoking status (P=0.14), and ATG use (P=0.03) were associated with CVEs.

| Table 1. Characteristics of patients according to treatment status |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Variable                  | Non-ATG–Treated Patients (n=219) | ATG-Treated Patients (n=347) | P Value |
| Age (yr)                  | 51±15                        | 44±12                       | <0.001 |
| Men (%)                   | 149 (68)                     | 219 (63)                    | 0.27   |
| Dialysis duration         | 12±11                        | 18±16                       | 0.15   |
| ESRD                      |                              |                             |        |
| Chronic GN                | 49 (22)                      | 73 (21)                     | 0.71   |
| APKD                      | 32 (15)                      | 48 (14)                     | 0.80   |
| Diabetes                  | 18 (8)                       | 32 (9)                      | 0.68   |
| Other                     | 120 (55)                     | 194 (56)                    | 0.80   |
| Hepatitis C seropositivity| 6 (3)                        | 7 (2)                       | 0.58   |
| First transplant          | 204 (93)                     | 267 (77)                    | 0.001  |
| Preformed HLA antibodies (%) | 24 (11)                    | 108 (31)                    | 0.02   |
| Live donor                | 16 (7)                       | 20 (6)                      | 0.46   |
| HLA mismatch number       | 4.4±1                        | 4.2±1.1                     | 0.03   |
| Past history of CVD       | 34 (16)                      | 24 (7)                      | 0.04   |
| Diabetes mellitus         | 19 (9)                       | 35 (10)                     | 0.58   |
| Hypertension              | 181 (83)                     | 278 (80)                    | 0.45   |
| Tobacco consumption       | 48 (22)                      | 87 (25)                     | 0.39   |
| Hypercholesterolemia      | 84 (39)                      | 104 (30)                    | 0.003  |
| Body mass index           | 24.2±4.6                     | 23.8±4.8                    | 0.08   |
| Acute rejection           | 46 (21)                      | 83 (24)                     | 0.42   |
| CMV exposed               | 126 (57)                     | 214 (62)                    | 0.33   |
| CMV viremia               | 30 (14)                      | 73 (21)                     | 0.03   |

Data are presented as the mean±SD or n (%) unless otherwise indicated.
Younger age positively impacted the effect of ATG on CVEs (hazard ratio [HR], 2.33; 95% confidence interval [95% CI], 1.34 to 4.07; P = 0.003; test for interaction, P = 0.01). Young patients (first and second tertiles, aged 41 and 55 years, respectively) who received ATG carried a similar risk than patients aged 20 years. There was a trend toward an effect of ATG on CVE in older patients (third tertile, aged 55 years). Nevertheless, the difference did not reach significance (HR, 1.82; 95% CI, 0.79 to 4.19; P = 0.16) (Table 3).

Cox regression analysis revealed that age (HR, 1.04; 95% CI, 1.02 to 1.06, for each increase in 1 year; P < 0.001), a past history of CVD (HR, 1.72; 95% CI, 1.11 to 3.60; P = 0.02), smoking status (HR, 1.74; 95% CI, 1.04 to 2.92; P = 0.04), and ATG use (HR, 2.36; 95% CI, 1.35 to 4.13; P = 0.003) were independent risk factors for CVEs (Table 4).

Overall model fit (using Hosmer–Lemeshow test) indicated a chi-squared value of 23 (P < 0.001). The area under the curve was 0.61 (range, 0.57–0.65) without ATG use and 0.66 (range 0.62–0.70) with the addition of ATG use (P = 0.07). The difference was 5%±2.7%.

We tested the effect of ATG on EA in different patient categories (e.g., past history of cardiovascular disease, acute rejection, delayed graft function). Among individuals who did not develop these complications, the risk of CVEs in the ATG-treated group remained significantly elevated in patients treated with ATG compared with those who were not (data not shown).

**Death and CVEs**
Seventy-three patients died during the study period. ATG use was not associated with death (HR, 1.04; 95% CI, 0.65 to 1.67; P = 0.86).

There were 123 patients who had a CVE or died during follow-up. ATG use was not predictive of this composite endpoint (HR, 1.28; 95% CI, 0.88 to 1.87; P = 0.19).

**Propensity Score Analyses**
First, propensity score was introduced in the model as a new variable. ATG use remained strongly associated with CVEs.
Table 3. Relative risk estimates of CVEs

<table>
<thead>
<tr>
<th>Category</th>
<th>HR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATG, unadjusted model</td>
<td>1.79 (1.05 to 3.06)</td>
<td>0.03</td>
</tr>
<tr>
<td>ATG, adjusted model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>2.33 (1.34 to 4.07)</td>
<td>0.003</td>
</tr>
<tr>
<td>Sex</td>
<td>1.80 (1.06 to 3.08)</td>
<td>0.03</td>
</tr>
<tr>
<td>Past history of CVD</td>
<td>1.92 (1.12 to 3.30)</td>
<td>0.02</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.80 (1.06 to 3.08)</td>
<td>0.03</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>1.95 (1.14 to 3.35)</td>
<td>0.02</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.80 (1.06 to 3.08)</td>
<td>0.03</td>
</tr>
<tr>
<td>Smoking status</td>
<td>1.75 (1.03 to 2.99)</td>
<td>0.04</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.78 (1.04 to 3.05)</td>
<td>0.04</td>
</tr>
<tr>
<td>HLA mismatch</td>
<td>1.86 (1.09 to 3.18)</td>
<td>0.02</td>
</tr>
<tr>
<td>First transplantation</td>
<td>1.79 (1.04 to 3.07)</td>
<td>0.04</td>
</tr>
<tr>
<td>Preformed HLA antibody</td>
<td>1.86 (1.09 to 3.17)</td>
<td>0.02</td>
</tr>
<tr>
<td>CMV exposition</td>
<td>1.76 (1.03 to 3.01)</td>
<td>0.04</td>
</tr>
<tr>
<td>Study period</td>
<td>1.82 (1.07 to 3.13)</td>
<td>0.03</td>
</tr>
<tr>
<td>ATG, multivariate-adjusted model</td>
<td>2.36 (1.35 to 4.13)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Relative risk estimates are derived from proportional hazards modeling with unadjusted ATG, individually adjusted ATG, and with multivariate adjusted ATG.

Table 4. Cox model of HRs of atherosclerotic events

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.04 (1.02 to 1.06)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.39 (0.82 to 2.37)</td>
<td>0.23</td>
</tr>
<tr>
<td>Past history of CVD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.72 (1.11 to 3.60)</td>
<td>0.02</td>
</tr>
<tr>
<td>ATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.36 (1.35 to 4.13)</td>
<td>0.003</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.74 (1.04 to 2.92)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

(HR, 1.96; 95% CI, 1.09 to 3.55; P=0.03). Mean follow-up was similar in the two groups (77±52 versus 70±67 months; P=0.17).

Second, we performed propensity score match analysis. Results obtained in this subcohort recapitulated those obtained from the overall cohort. The cumulative incidence of CVEs was higher in ATG-treated patients (18% versus 9%; P=0.02) (Figure 1B).

ATG remained a significant risk factor for CVEs (HR, 2.09; 95% CI, 1.11 to 3.98; P=0.02). Age, past history of CVD, and smoking status were also associated with CVEs.

Because only ATG was used in the earliest period, we performed a second analysis excluding these patients. There were 137 patients included in the second analysis; the results were quite similar and ATG was still associated with CVEs (HR, 2.12; 95% CI, 1.12 to 4.01; P=0.02).

**Immune Activation**

**Expansion of Activated CD8+ T Cell in ATG-Treated Patients**

Total CD8+ T-cell count did not vary during the first year after transplantation. Late-stage differentiated CD8+ T cells increased 1 year after transplantation in ATG-treated patients (CD8+CD28-: 156±111 [49%±22%] versus 262±189 [57%±23%], P=0.04; and CD8+CD57+CD28-: 75±21 [23%±9%] versus 140±121 [32%±14%], P=0.02), whereas naïve CD8+ T cells significantly decreased 1 year after transplantation (CD8+CD28+: 164±116 [53%±12%] versus 115±80 [42%±13%], P=0.02; and CD8+CD57+CD28+: 160±113 [52%±20%] versus 109±81 [41%±22%], P=0.01). These modifications were amplified in CMV-exposed patients (data not shown). All CD8+ T-cell subtypes remained unchanged in patients who received α-CD25 mAb induction therapy.

**Influence of CMV Infection on ATG-Associated Atherosclerosis**

Patients were divided into three groups according to CMV status: CMV-naïve patients (n=226), CMV-exposed patients without CMV replication (n=237), and CMV-exposed patients with CMV replication (n=103).

Because CMV infection amplifies CD8+ T-cell activation in ATG-treated patients, we studied the effect of ATG on EA in CMV-naïve and CMV-exposed patients. ATG was not associated with CVEs in CMV-naïve patients (HR, 1.29; 95% CI, 0.54 to 3.06; P=0.57). By contrast, CMV-exposed patients who received ATG had an increased risk of CVEs compared with those who did not receive ATG (HR, 2.12; 95% CI, 1.06 to 4.24; P=0.02). This effect remained after full adjustment (HR, 2.57; 95% CI, 1.26 to 5.26; P=0.01). The results of tests for interaction were significant (P=0.003).

We also compared the effect of CMV replication on the occurrence of CVE in ATG-treated patients and in non-ATG-treated patients (Figure 2). Whereas CMV viremia was significantly associated with CVE in ATG-treated patients (HR, 2.07; 95% CI, 1.16 to 3.70; P=0.01), we did not observe any effect in non-ATG-treated patients (HR, 1.49; 95% CI, 0.42 to 5.25; P=0.54). The association remained after adjustment for other parameters in ATG-treated patients (HR, 1.91; 95% CI, 1.08 to 3.39; P=0.03).

**DISCUSSION**

In this study, we showed that the risk of CVEs increased among patients who received ATG compared with those who received anti-CD25 mAb. More precisely, ATG-treated patients had a 2-fold increased risk of CVEs. The introduction of ATG in the predictive model significantly improves its explanatory value. Similar results derived from propensity score-matched analyses support these findings. Our results also suggest that ATG-induced lymphocyte activation may promote atherosclerosis and that this effect could be amplified by CMV infection.
There is a growing amount of evidence demonstrating that CD8+ T-cell activation promotes atherosclerosis. Major advances in knowledge come from studies in HIV-infected individuals. Recent data showed that a higher frequency of activated CD8+ T cells among HIV-infected patients was associated with subclinical carotid disease. An expansion of CD28−CD57+CD8+ T cells has also been described in patients with coronary artery disease compared with healthy individuals. Finally, CD8+ T-lymphocyte depletion by anti-CD8 mAb in apoE-deficient mice fed a high-fat diet ameliorates atherosclerosis and infusion of CD8+ T lymphocytes promotes the development of vulnerable atherosclerotic plaques in lymphocyte-deficient apoE−/− mice. Such T cells produce high amounts of proinflammatory cytokines, including IFN-γ and TNF-α. Indirect evidence suggests that ATG-induced atherosclerosis is a result of CD8+ T-cell activation. First, we showed that ATG use led to the expansion of activated CD8+CD28− T cells. Second, the effect of ATG on CVEs seems to be restricted to CMV-exposed patients in whom ATG-induced CD8+ T-cell expansion is greater. Third, we recently reported that CD8+ T-cell depletion partly prevents the progression of atherosclerotic plaques in skin allograft apoE−/− mice (D. Ducloix et al., unpublished observations). All together, these results suggest that CD8+ T cells could mediate ATG-induced vascular damage.

We recently reported that both CMV exposure and infection increased the incidence of post-transplant CVEs. Our present results suggest that the effect of CMV seems to be limited to ATG-treated patients. CMV could act as a trigger for CD8+ T lymphocyte activation and proliferation, thereby contributing to increase immune-mediated atherosclerotic process. It has been shown that most of the late-differentiated CD8+ T cells are part of large clonal expansions that are specific for persistent viruses, mainly CMV. This suggests that repeated immune challenges in the context of ATG-induced lymphopenia-induced proliferation could drive a proinflammatory response, which promotes and/or aggravates atherosclerosis.

Immune reconstitution after lymphocyte depletion by ATG is a complex phenomenon. CD8+ activation is a characteristic feature of immune reconstitution and compelling data suggest its role in atherosclerosis. Nevertheless, some other ATG-induced immune alterations may have a role in post-transplant atherosclerosis. Lymphocyte depletion with ATG can be complicated by systemic coagulation activation. Indeed, ATG activates tissue factor procoagulant activity on monocytic cells. Nevertheless, this early effect is unlikely to explain the late burden of CVEs after ATG treatment. Infections are more frequent in ATG-treated patients and a large number of infectious agents have been linked with an increased risk of CVD. Thus, CMV disease is more frequent after ATG and is associated with post-transplant CVEs. An ATG-induced infectious burden might alternatively contribute to atherosclerosis. ATG also induces B-cell depletion. Interestingly, even when the effect of B cells on atherosclerosis is subset dependent, B-cell depletion has been associated with atherosclerosis progression. Compelling data from both animal models and other human populations suggest CD8+ activation as the trigger of ATG-induced atherosclerosis. Nevertheless, immune reconstitution could promote a more general proatherogenic milieu.

Randomized studies proved ATG to be superior to anti-CD25 mAb in renal transplant recipients with high immunologic risk. In such patients, benefits likely overcome the increase in the risk of CVEs. However, in our view, these patients should be considered at very high risk of cardiovascular complications and taken over in this way. By contrast, the superiority of ATG in low-to-moderate immunologic risk patients is more questionable. Indeed, whereas the rate of acute rejection is not reduced by ATG use in those patients, infections are more frequent leading to a worse benefit/risk ratio. Present results add a new risk in using ATG. Evaluation of cardiovascular risk should at least in part contribute to the decision of whether to use ATG. Cardiovascular risk support should be reinforced in ATG-treated patients.

The absence of randomization is a limitation of our study. Nevertheless, the introduction of such a randomized study is unlikely. Considering our results, 400 patients should be included and followed for at least 10 years to confirm the observed difference in the cumulated incidence of CVEs between patients who received ATG and those who did not. Registry studies have the potential to include a large number of patients, but often lack accurate confounding factors analysis. In our view, our study offers the best compromise between an unfeasible randomized trial and an uninterpretable registry study. Patients who received ATG differed in many ways from those who did not. Propensity score analysis takes these differences into account and minimizes potential confounding by indication. Although our study is retrospective, there were no missing data and no patients were lost to follow-up. Therefore, we feel that our study has the
capacity to conclude that ATG use is associated with an increased risk of CVEs in kidney transplant patients. Our study suggests that ATG promotes post-transplant accelerated atherosclerosis. ATG-induced immune activation could explain this association. Further studies should confirm this hypothesis.

**CONCISE METHODS**

Complete methods are available in the Supplemental Material.

**Study Design and Population**

We analyzed a prospective cohort of 566 consecutive renal transplant recipients who had a kidney transplant at the transplant unit of the University Hospital of Besançon between January 1993 and December 2009. The ethics committee of Franche-Comté approved this study.

All patients received quadruple sequential immunosuppression. Induction consisted of either ATG (Fresenius) or thymoglobulin (Genzyme) \((n=347, 61\%)\) or anti-CD25 mAb basiliximab (Novartis) \((n=219, 39\%)\).

ATG was used in all patients until April 1998 \((n=118)\). From May 1998 to May 2004, basiliximab use was reserved for patients aged \(>59\) years \((n=215)\). From June 2005, ATG was restricted to patients with a second transplant and patients with a panel reactive antibody \(>20\%\) regardless of age \((n=233)\).

The same maintenance immunosuppressive treatments were used, including cyclosporine (January 1993 to July 2001), tacrolimus (August 2001 to December 2009), azathioprine (January 1993 to October 2000), or mycophenolate mofetil (November 2000 to December 2009), and steroids.

All patients except CMV seronegative recipients of a CMV seronegative donor received CMV prophylaxis with valacyclovir in the first 3 months after transplantation.

Characteristics of the study population are described in Tables 1 and 2.

**CMV Infection and Disease**

Patients were considered to have CMV infection in any case of positive PCR. CMV was defined by the need for treatment in a patient with viral replication. CMV exposure was defined by positive pretransplant CMV serology and/or post-transplant CMV infection or disease.

**Confounding Factors**

Age, sex, weight, size, hemodialysis duration before transplantation, pretransplant CVD, diabetes mellitus, hypercholesterolemia, hypertension, body mass index, smoking status, panel reactive antibody, rank of transplantation (first versus iterative), donor type, and immunosuppressive treatment (type of induction, cyclosporine versus tacrolimus, azathioprine versus mycophenolate mofetil) were assessed.

**Atherosclerotic Events**

Atherosclerotic events were considered as previously reported.8

---

**Coronary Heart Disease**

Myocardial infarction was documented by serial 12-lead electrocardiogram evidence or Q-wave infarction and appropriate myocardial enzyme elevations, coronary revascularization including coronary artery bypass surgery or percutaneous transluminal coronary angioplasty, or typical history of angina with abnormal coronaryography.

**Stroke/Cerebrovascular Disease**

Both nonhemorrhagic and hemorrhagic strokes were confirmed by neurologic examination findings consistent with new-onset focal neurologic deficits, with or without computed tomography or magnetic resonance imaging evidence of cerebral infarction, or symptomatic extracranial artery stenosis resulting in carotid endarterectomy.

**Abdominal Aortic or Lower Extremity Arterial Disease**

Doppler or arteriography findings were used to confirm the presence of abdominal aortic repair, lower extremity revascularization via bypass surgery or angioplasty, lower extremity amputation, or new onset of intermittent claudication.

Two physicians independent of the study were responsible for diagnostic ascertainment. This analysis was performed without knowledge of baseline characteristics.

**Lymphocyte Subsets**

Lymphocyte subsets were studied in 62 random patients included in the ORLY-EST study (Influence de l’Orientatie de la Réponse Lymphocytaire dans l’Athérosclérose Post-Transplantation). Briefly, ORLY-EST, started in November 2008, is an observational study including all incident RTRs in seven French transplant centers (Strasbourg, Nancy, Reims, Dijon, Clermont-Ferrand, Kremlin-Bicêtre, and Besançon). Blood samples are collected at transplantation and 1 year after transplantation, and were sent with written consent to the Biomonitoring Platform (CIC-BT506, EFS Besançon, France) for processing and storage. Sixty-two patients were extracted from the main cohort to explore immune exhaustion. The ethics committee of Franche-Comté approved this study (2008).

Absolute numbers of circulating B and T cells (CD4+ and CD8+) were determined on a FC500 cytometer (Beckman Coulter, Villepinte, France) as previously described.5,9

**Statistical Analyses**

**Baseline Characteristics**

Arithmetic mean was calculated and expressed as the mean±SD. We separated patients who received ATG from those who received anti-CD25 mAb. We first compared baseline characteristics using two-sample \(t\) tests, Wilcoxon rank-sum tests, or chi-squared tests as appropriate.

**Propensity Score Analyses**

Because this was a nonrandomized study, we supposed that there were inherent differences between the two groups. To overcome these limitations, we used propensity scores to parsimoniously adjust for confounding factors and to address potential confounding by indication. By using propensity scores, better control for the likelihood
of being assigned to a group is expected and occult biases are reduced. We calculated the propensity score of receiving ATG after fitting a multivariable logistic regression model with ATG use as the dependent variable. Data that could affect the choice of immunosuppressive agents were used to calculate the propensity score and included age, pretransplant history of malignancy, duration of dialysis before transplant, pretransplant anti-HLA antibodies, number of HLA mismatches, donor type, cold ischemia time, and period of transplant (1993–2001, 2001–2004, 2004–2008). Pretransplant traditional cardiovascular risk factors (sex, previous cardiovascular disease, hypercholesterolemia, hypertension, body mass index, smoking status, and diabetes) were also included in the model. We matched one ATG-treated patient to one non-ATG–treated patient by their propensity score + 0.02 (0.1×SD of the propensity score) to generate a subcohort of 159 patients who were treated with ATG and 159 patients who were not.

Survival Analyses
Using log rank tests on Kaplan–Meier nonparametric estimates of the survival without death-censored CVE distribution, we selected variables with a P value ≤0.20. The selected variables were included into a Cox proportional hazards model and a backward stepwise selection process was performed, this time at a classic α=0.05. Because sex and age were potential confounding variables, they were also entered into the Cox model regardless of the significance of their relationships with death. Tobacco consumption was accounted for as currently smoking versus nonsmoking definition variables. Because indications for the use of ATG differed during the study period, we defined three periods (1993–2001, 2001–2004, 2004–2008) corresponding to different clinical practices. The overall presence of interactions between ATG and different parameters was evaluated by the Wald test. The propensity score was used in two ways. First, the propensity score was forced in a second Cox model in order to reduce selection bias. Second, survival analysis was performed in a subcohort of ATG-treated and non-ATG–treated patients matched by their propensity score.

ACKNOWLEDGMENTS
This study is supported by grants from the Fondation de Transplantation (ET-031211 and ET-050320 to C.C.).

DISCLOSURES
None.

REFERENCES

This article contains supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2013060663/-/DCSupplemental.
Patients and Methods

Study design and populations

We analysed a prospective cohort of 566 consecutive RTR having received a kidney transplant at the transplant unit of the University hospital of Besançon between January 1993 and December 2009. The ethic committee of Franche Comté has approved the study. All the patients received a quadruple sequential immunosuppression. Induction consisted of either ATG (n=347, 61%) [ATG Fresenius® (day 0: 9 mg/kg; days 1-4: 3 mg/kg/d, n= 221, 64%) or Thymoglobulin® (Genzyme) (day 0: 2 mg/kg; days 1-4: 1 mg/kg/d, n= 126, 36%)] or monoclonal anti-CD25 antibody (n=219, 39%) [Simulect® (Novartis) (day 0: 20mg, day 4: 20mg)].

ATG was used in all patients until April 1998 (n=118). From May 1998 to May 2004, Simulect use was reserved for patients older than 59 years (n=215). From June 2005, ATG was restricted to second transplant and patients with PRA > 20% regardless of age (n=233).

The same maintenance immunosuppressive treatments were used including Cyclosporine (January 1993-July 2001) or Tacrolimus (August 2001-December 2009), Azathioprine (January 1993-October 2000) or Mycophenolate Mofetil (November 2000-December 2009), and steroids.

All the patients except CMV seronegative recipients of a CMV seronegative donor received CMV prophylaxis with Valaciclovir in the first three months following transplantation. Antiviral prophylaxis dose was adapted to renal function. All patients received Pneumocystis antimicrobial prophylaxis with trimethoprim-sulfamethoxazole.

Characteristics of the study population are described in tables 1 and 2.

CM infection and disease

CMV serology (ELISA) was performed before transplantation. Donor CMV serology was assessed through medical records. CMV PCR were performed weekly until months three post-transplant, monthly until six months post-transplant, and each year during follow-up.
Patients were considered to have CMV infection in any case of positive PCR. CMV disease was defined by the need of treatment in a patient with viral replication. CMV exposure was defined by a positive pre-transplant CMV serology and/or post-transplant CMV infection or disease.

**Confounding factors**
Age, gender, weight, size, hemodialysis duration before transplantation, pre-transplant cardiovascular disease, diabetes mellitus, hypercholesterolemia, hypertension, body mass index, smoking status, panel reactive antibody, rank of transplantation (first vs iterative), donor type, and immunosuppressive treatment (type of induction, cyclosporine vs tacrolimus, azathioprine vs mycophenolate mofetil) were assessed.

**Atherosclerotic events**
*Coronary heart disease*: Myocardial infarction documented by serial 12-lead electrocardiogram evidence or Q-wave infarction and appropriate myocardial enzyme elevations; coronary revascularization including coronary artery bypass surgery or percutaneous transluminal coronary angioplasty; typical history of angina with abnormal coronarography.
*Stroke/cerebrovascular disease*: Both nonhemorrhagic and hemorrhagic strokes confirmed by neurologic examination findings consistent with new onset focal neurologic deficits, with or without computed tomography or magnetic resonance imaging evidence of cerebral infarction; symptomatic extracranial artery stenosis resulting in carotid endarterectomy.
*Abdominal aortic or lower extremity arterial disease*: Abdominal aortic repair; lower extremity revascularization via bypass surgery or angioplasty; lower extremity amputation; new onset of intermittent claudication confirmed by doppler or arteriography findings.

Two physicians independent of the study were responsible for diagnostic ascertainment. This analysis was performed without knowledge of baseline characteristics.
**Lymphocyte subsets**

Lymphocyte subsets were studied in 62 patients included in the ORLY-EST study (Influence de l’Orientation de la Réponse Lymphocytaire dans l’athérosclérose post-transplantation). Briefly, ORLY-EST, started in November 2008, is an observational study including all incident RTR in seven French transplants centers (Strasbourg, Nancy, Reims, Dijon, Clermont-Ferrand, Kremlin-Bicêtre, Besançon). Blood samples are collected at transplant and one year after transplantation, and sent with written consent to the Biomonitoring Plateform (CIC-BT506, EFS Besançon, France) for processing and storage. To date, five hundred patients have been included in this study. Sixty-two patients were extracted from the main cohort to explore immune exhaustion. The ethic committee of Franche-Comté approved the study (2008).

Absolute numbers of circulating B and T cells (CD4+ and CD8+) were determined on FC500 cytometer (Beckman Coulter, Villepinte, France) as previously described (8, 9). Naive CD4+ T cells were also assessed as CD45RA+, CD62L+, CD45RO– CD4+ CD3+ T cells using the following antibodies: FITC-conjugated CD45RA (clone HI100), phycoerythrin-CD62L (Dreg56) (BD Biosciences, Le Pont de Claix, France), ECD-CD45RO (UCHL1), PC7-CD4 (13B8.2) and allophycocyanin-CD3 (UCHT1) (Beckman Coulter) (9). Exhausted T cells were assessed as CD57+CD28– using the following antibodies: FITC-conjugated CD57 (NC1) (Beckman Coulter), PerCP/Cy5.5 CD28 (L293) (BD Biosciences). These T cell subsets were analyzed on FACS CANTO II (BD Biosciences) flow cytometer.

**Statistical analysis**

**Baseline characteristics**

Arithmetic mean was calculated and expressed as ± SD.

We separated patients who had received ATG from those who had received anti-CD25 mab. We first compared baseline characteristics using two-sample t-tests, Wilcoxon rank-sum test, or chi-2 test as appropriate.

**Propensity score analyses**
As this was a nonrandomized study, we supposed that there were inherent differences between the two groups. To overcome these limitations, we used propensity scores to parsimoniously adjust for confounding factors and to address potential confounding by indication. By using propensity scores, a better control for the likelihood of being assigned to a group is expected and occult biases are reduced (23). We calculated the propensity score of receiving ATG after fitting a multivariable logistic regression model with ATG use as the dependent variable. Data that could affect the choice of immunosuppressive agents were used to calculate the propensity score and included age, pre-transplant history of malignancy, duration of dialysis prior to transplant, pre-transplant anti-HLA antibodies, number of HLA mismatches, donor type, cold ischemia time, period of transplant (1993-2001 / 2001-2004 / 2004-2008). Pre-transplant traditional cardiovascular risk factors (gender, previous cardiovascular disease, hypercholesterolemia, hypertension, BMI, smoking status, diabetes) were also included in the model. We matched 1 ATG-treated patient to 1 non-ATG-treated patient by their propensity score + 0.02 (0.1 x standard deviation of the propensity score) to generate a sub-cohort of 159 ATG-treated patients and 159 non-ATG-treated patients.

Survival analyses

Using log rank tests on Kaplan Meier nonparametric estimates of the survival without death-censored AE distribution, we selected variables with a p value lower than, or equal to, 0.20. The selected variables were included into a Cox proportional hazards model, and a backward stepwise selection process was performed, this time at a classical α=0.05. Gender and age being potential confounding variables, they were also entered into the Cox model, no matter the significance of their relationships with death. Tobacco consumption was accounted for as currently smoking versus non-smoking definition variables. Because indications for the use of ATG differed during the study period, we defined 3 periods (1993-2001/2001-2004/2004-2008) corresponding to different clinical practices.
Propensity score was used in two ways. First, propensity score was forced in a second Cox model in order to reduce selection bias. Second, survival analysis was performed in a sub-cohort of ATG-treated and non-ATG-treated patients matched by their propensity score. Results are expressed as hazard ratio (HR) and 95% confidence interval (CI), with a \( p \) value testing the null hypothesis: HR=1. Therefore when \( p \) value is less than 0.05, HR is significantly different from 1, either greater than 1 (i.e. risk of death is increased) or less than 1 (i.e. risk of death is decreased). Assumptions of Cox models (log-linearity, proportionality of risk in time) were met in this analysis.