Pretransplant thymic function predicts acute rejection in antithymocyte globulin–treated renal transplant recipients

Jamal Bamoulid[1,2,3,4], Cécile Courivaud[1,2,3,4], Thomas Crepin[1,2,3,4], Clémence Carron[1,2,3], Emilie Gaiffe[4,5], Caroline Roubiou[2,3,4], Caroline Laheurte[1,6], Bruno Moulin[7], Luc Frimat[8], Philippe Rieu[9], Christiane Mousson[10], Antoine Durrbach[11], Anne-Elisabeth Heng[12], Jean-Michel Rebibou[1,10], Philippe Saas[1,2,3,5,6] and Didier Ducloux[1,2,3,4,5]

[1]INSERM, UMR1098, Federation hospitalo-universitaire INCREASE, Besançon, France; [2]Faculté de Médecine et de Pharmacie, University Bourgogne Franche-Comté, Besançon, France; [3]Structure Fédérative de Recherche, Besançon, France; [4]Department of Nephrology, Dialysis, and Renal Transplantation, CHU Besançon, Besançon, France; [5]CHU Besançon, CIC Biothérapie, INSERM CIC1431, Besançon, France; [6]EFS Bourgogne Franche-Comté, Plateforme de Biomonitoring, CIC 1431/UMR1098, Besançon, France; [7]Department of Nephrology, Dialysis, and Renal Transplantation, CHU Strasbourg, Strasbourg, France; [8]Department of Nephrology, Dialysis, and Renal Transplantation, CHU Nancy, Nancy, France; [9]Department of Nephrology, Dialysis, and Renal Transplantation, CHU Reims, Reims, France; [10]Department of Nephrology, Dialysis, and Renal Transplantation, CHU Dijon, Dijon, France; [11]Department of Nephrology, Dialysis, and Renal Transplantation, CHU Kremlin-Bicêtre, Le Kremlin-Bicêtre, France; and [12]Department of Nephrology, Dialysis, and Renal Transplantation, CHU Clermont-Ferrand, Clermont-Ferrand, France

Lack of clear identification of patients at high risk of acute rejection hampers the ability to individualize immunosuppressive therapy. Here we studied whether thymic function may predict acute rejection in antithymocyte globulin (ATG)-treated renal transplant recipients in 482 patients prospectively studied during the first year post-transplant of which 86 patients experienced acute rejection. Only $CD45RA^+CD31^+CD4^+$ T cell (recent thymic emigrant [RTE]) frequency (RTE%) was marginally associated with acute rejection in the whole population. This T-cell subset accounts for 26% of $CD4^+$ T cells. Pretransplant RTE% was significantly associated with acute rejection in ATG-treated patients (hazard ratio, 1.04; 95% confidence interval, 1.01–1.08) for each increased percent in RTE/$CD4^+$ T cells), but not in anti-CD25 monoclonal (αCD25 mAb)-treated patients. Acute rejection was significantly more frequent in ATG-treated patients with high pretransplant RTE% (31.2% vs. 16.4%) or absolute number of RTE/mm^3 (31.7 vs. 16.1). This difference was not found in αCD25 monclonal antibody–treated patients. Highest values of both RTE% (>31%, hazard ratio, 2.50; 95% confidence interval, 1.09–5.74) and RTE/mm^3 (>200/mm^3, hazard ratio, 3.71; 95% confidence interval, 1.59–8.70) were predictive of acute rejection in ATG-treated patients but not in patients having received αCD25 monoclonal antibody). Results were confirmed in a retrospective cohort using T-cell receptor excision circle levels as a marker of thymic function. Thus, pretransplant thymic function predicts acute rejection in ATG-treated patients.

Kidney International (2016) **89,** 1136–1143; http://dx.doi.org/10.1016/j.kint.2015.12.044

KEYWORDS: acute graft rejection; CMV; immune senescence; thymus; transplantation

Although its rate has been considerably reduced during the past decades, acute rejection remains a frequent and serious complication affecting long-term graft survival.[1,2] Indeed, about 10% to 20% of kidney transplant recipients experience at least 1 episode of acute rejection. Identification of pretransplantation risk factors may help to explain why acute rejection only develops in some individuals, even though all are exposed to similar transplantation immunosuppression. Moreover, identification of high-risk patients may help to individualize treatments. However, clinical trials testing prevention strategies may have greater power if conducted among patients at highest risk. Current strategies use clinical factors (donor/recipient age and race), human leukocyte antigen typing, and alloantibody screening for risk stratification. These approaches are neither accurate nor sensitive. Nevertheless, to date few other pretransplantation biomarkers have been identified.

Augustine *et al.*[3] and Poggio *et al.*[4] reported that patients with positive donor-reactive or panel T-cell–reactive interferon-gamma Enzyme-Linked ImmunoSpot assays (Cellular Technology Limited, Shaker Heights, OH) were more likely to experience acute rejection. Although results are controversial, some studies suggest that pretransplantation soluble CD30 could be predictive of acute rejection.[5] Finally,

Correspondence: *Didier Ducloux, Department of Nephrology, Dialysis, and Renal Transplantation, F-25030, Besançon, France. E-mail: dducloux@chu-besancon.fr*

Received 3 August 2015; revised 26 November 2015; accepted 17 December 2015; published online 2 March 2016

different genetic polymorphisms have been associated with acute rejection.[6–8] All these biomarkers suffer, at different levels, from lack of accuracy, poor clinical transfer feasibility, or weak reproducibility.

Involution of the thymus with age is associated with a decline in naive T-cell output. This is thought to contribute to the reduction in T-cell diversity observed in elderly individuals and to a decreased ability of the immune system to generate antigen-specific responses against pathogens and vaccines. This suggests that the altered thymic activity is the founding event of the decline of immune function in older individuals.[9]

Interestingly, we recently reported that pretransplantation lymphocyte phenotype considerably influences post-antithymocyte globulin (ATG) immune reconstitution.[10] More precisely, after transplantation we found reduced thymic output, lymphocyte exhaustion, and T regulatory (Treg) expansion in ATG-treated patients with poor pretransplantation thymic function.[10] Such changes were not observed in anti-CD25 monoclonal antibody (αCD25-mAb)-treated patients. It could be speculated that poor pretransplantation thymic function predicts greater posttransplantation immunosuppression in ATG-treated patients. Conversely, those with good thymic function may have a greater risk of acute rejection and require intensified immunosuppression.

To test this hypothesis, we conducted a prospective multicenter study to assess whether pretransplantation thymic function may predict acute rejection in ATG-treated patients. We used an independent retrospective cohort to validate our results.

RESULTS

Study population

Characteristics of the study population are depicted in Table 1.

Table 1 | Clinical characteristics of the study population

Variable	αCD25 monoclonal antibody (n = 352)	ATG (n = 157)	*P* value
Age (y)	52 ± 14	49 ± 12	0.036
Sex (% male)	68%	59%	0.139
Dialysis	92%	93%	0.710
Hemodialysis/peritoneal dialysis	82%/18%	85%/15%	0.469
Dialysis vintage (mo)	38 ± 35	42 ± 52	0.194
Pre-emptive transplantation	9%	7%	0.438
Diabetes	19%	19%	0.984
Dyslipidemia	34%	32%	0.620
Hypertension	85%	86%	0.811
Body mass index (kg/m^2)	23.9 ± 4.1	24.2 ± 4.4	0.541
First transplant	92%	89%	0.292
Human leukocyte antigen mismatches	4.1 ± 1.2	4.3 ± 1	0.072
Percentage of immunized patients	18%	52%	<0.001
Positive CMV serologic test results	52%	64%	0.061
% CMV D$^+$/R$^-$	25%	22%	0.181

ATG, antithymocyte globulin; CMV cytomegalovirus.

Thymic function

Both CD45RA$^+$CD31$^+$ CD4$^+$ T cells (recent thymic emigrant [RTE]/mm^3) and RTE frequency (RTE%; percentage of CD45RA$^+$CD31$^+$ CD4$^+$ T cells among CD4$^+$ T cells) were abnormally distributed. The median value of RTE was 157/mm^3 (range, 6–712/mm^3). This T-cell subset accounts for 26% (median; range, 2%–67%) of CD4$^+$ T cells before transplantation.

RTE decreased 1 year after transplantation (median post-transplantation value, 93/mm^3; range, 1–84/mm^3; $P < 0.001$). RTE remained stable in patients who received αCD25 mAb (138 vs. 156/mm^3 after and before transplantation, respectively; $P = 0.245$) but significantly decreased in those who received ATG (32 vs. 158/mm^3 after and before transplantation, respectively; $P < 0.001$). Similar results were observed with analysis of %RTE (24% vs. 26% after and before transplantation, respectively, in αCD25 mAb-treated patients and 15% vs. 26% after and before transplantation, respectively, in ATG-treated patients; $P < 0.001$). Thus, %RTE and absolute number of RTE/mm^3 decreased in ATG-treated patients, in contrast to αCD25-mAb–treated patients.

The frequency of RTE among CD4$^+$ T cells was inversely related to age ($r = -0.23$; $P < 0.001$) (Figure 1a) and was significantly lower in men (25% vs. 31% in women; $P < 0.001$) (Figure 1b). Finally, %RTE weakly depends on dialysis duration ($r = -0.11$; $P = 0.071$). Similar results were observed for the absolute number of RTEs/mm^3. %RTE was inversely related to the frequency of CD45R0$^+$CD4$^+$ T cells ($r = -0.81$; $P < 0.001$).

The %RTE was split into tertiles. Younger age ($P < 0.001$), female sex ($P = 0.005$), and shorter duration of dialysis ($P = 0.017$) were associated with the highest values of %RTE, as well as absolute values of RTE.

Acute rejection

Eighty-six patients (17.8%) experienced at least 1 episode of acute cellular rejection.

No clinical or biological parameters were associated with acute rejection. Nevertheless, %RTE was marginally associated with acute rejection (hazard ratio [HR], 1.02; 95% confidence interval [CI], 0.99–1.04 for each increase of 1% in RTE/CD4$^+$ T cells; $P = 0.071$). As scheduled, we considered separately patients who received ATG and those who did not receive ATG. The rate of acute rejection was similar in ATG-treated patients and in those who received αCD25 mAb (21% vs. 15%; $P = 0.266$). However, graft and patient survival did not differ (92% vs. 93%; $P = 0.612$ and 98% vs. 97%; $P = 0.928$, respectively). %RTE was not associated with acute rejection in α-CD25 mAb–treated patients (HR, 1.01; 95% CI, 0.98–1.04 for each increase of 1% in RTE/CD4$^+$ T cells; $P = 0.478$). By contrast, %RTE predicted acute rejection in ATG-treated patients (HR, 1.04; 95% CI, 1.01–1.08 for each increase of 1% in RTE/CD4$^+$ T cells; $P = 0.032$).

The area under the receiver operating characteristic curve was 0.58 (95% CI, 0.55–0.61; $P = 0.023$) in the whole