Kidney International, Vol. 60 (2001), pp. 341–349

Multicenter trial of one HLA-DR–matched or mismatched blood transfusion prior to cadaveric renal transplantation

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Background. The beneficial effect of blood transfusions before cadaveric renal transplantation on allograft survival, although previously well documented, has become controversial in light of their adverse effects. Recently, it has been suggested that their clinical benefits are due to HLA-DR sharing between the blood donor and recipient.

Methods. In this prospective study, 144 naive patients were randomly assigned to receive one unit of blood matched for one-HLA-DR antigen ($N = 49$), or one unit of mismatched blood ($N = 48$), or to remain untransfused ($N = 47$). Graft survival and acute rejection rate were analyzed in 106 cadaveric renal allograft recipients receiving the same immunosuppressive protocol.

Results. Graft survival was similar in the three groups at one and five years: 91.7 and 80% in untransfused patients, 90.3 and 79.3% in patients transfused with one DR-antigen–matched unit, and 92.3 and 83.7% in patients transfused with HLA-mismatched blood. The difference in the incidence of six-month post-transplant acute rejections was not statistically significant in the three groups: 12 out of 36, 33.3% in nontransfused patients; 6 out of 31, 19.4% in patients transfused with one DR-matched blood; and 13 out of 39, 33.3% in patients transfused with mismatched blood.

Conclusion. The results of our prospective randomized trial showed that in a population of naive patients, one transfusion mismatched or matched for one HLA-DR antigen given prior to renal transplantation had no significant effect on the incidence and severity of acute rejection, and did not influence overall long-term graft outcome. Considering the potentially deleterious adverse effects of blood transfusions, the costs, and the considerable logistical efforts required to select and type blood donors, such a procedure cannot be recommended in a routine practice for patients awaiting cadaveric kidney transplantation.

The history of the transfusion of blood is an essential part of the history and development of transplantation. When the modern era of renal transplantation started in the 1960s, the old concept of deleterious sensitization of the patient to foreign proteins persisted, leading clinicians managing dialysis patients to the absolute avoidance of blood products. This was the case until 1973, when Opelz found that untransfused cadaveric renal transplant recipients had a poor graft outcome and postulated the concept of a transfusion effect [1]. From this time, a flurry of clinical, experimental, and fundamental investigations confirmed the premise, and a stream of hypotheses successively attempted to explain the transfusion effect [2]. When cyclosporine (CsA) became the cornerstone of immunosuppression, most clinical studies reported the progressive disappearance of the improvement of renal allograft survival in transfused recipients [3]. First, the benefits of blood transfusions have been questioned as a result of improved graft survival in nontransfused patients due to the improvement in rejection

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Key words: kidney transplantation, graft survival, organ rejection, typing blood, transfusion policy, tolerogeneic effect.

Received for publication October 5, 2000
and in revised form February 9, 2001
Accepted for publication February 13, 2001

diagnosis, immunosuppressive therapy, and overall patient care [4]. Second, it has become evident that the detrimental effects of transfusions, that is, the risk of transmitting viral agents and the risk of sensitization, outweighed a hypothetical and still poorly understood immunologic effect. Finally, the availability of human recombinant erythropoietin has eliminated the need for iterative transfusions in severely anemic dialysis patients. These facts have led most centers in the United States, United Kingdom, and Scandinavia to reappraise their transfusion policy, while several European teams still advocate blood transfusion protocols as a means of inducing specific allograft tolerance [5]. In 1989, from retrospective data Lagaaij et al suggested that the degree of HLA class II antigen compatibility between the blood donor and the recipient might be crucial in the explanation of why a single transfusion led to immunization and graft rejection in some cases, while in others, it improved survival without an excessive risk of sensitization [6]. However, during the last decade, further controlled trials analyzing the effect of matched transfusions have yielded conflicting results [7–10]. Here we report the clinical results of the first prospective, multicenter randomized study on the effects of a single leukocyte-rich blood transfusion, either mismatched or matched for one HLA-DR antigen, compared with zero transfusion, conducted in a population of naive untransfused dialysis patients awaiting cadaveric renal transplantation.

METHODS

Trial design

This open, randomized, prospective trial was conducted in eight kidney transplantation centers from the Groupe Coopératif de Transplantation d'Ile de France (GCIF) in France. Approval of the local ethics committee was obtained before the start of the study. After giving their written informed consent, male or female patients undergoing dialysis and awaiting a first renal cadaver transplant were included. No age limit was required, and children were eligible for inclusion. Patients were randomly assigned either to receive one unit of DR-matched blood or one unit of DR-mismatched blood or to remain untransfused. Randomization was performed immediately after patient inclusion in a 1:1:1 fashion using sealed envelopes, and was stratified by the center. The information regarding DR compatibility between blood donor and recipient remained blinded for transfused patients and investigators.

Patients were excluded from the study if they had been previously transfused, if female patients previously had been pregnant, and if preformed anti-HLA antibodies (IgG or IgM) were found in the serum. Patients participating in another clinical trial were also excluded. Patients from the three groups (including those who were randomized to receive HLA-typed transfusions) who received additional transfusions as medically indicated were withdrawn from further analysis regarding the influence of DR-matched transfusions on graft outcome. Patients who received a living-related donor graft or patients who had received or were receiving another organ transplant other than a kidney, were also withdrawn. Finally, patients who decided to leave the study were withdrawn.

Transfusion protocol

Blood units were obtained from the local blood banks. The blood donors were selected from a panel of 300 blood donors who had volunteered for anonymous blood donation and had subsequently been HLA A-B-DR typed. The blood units were 220 to 350 mL leukocyte-rich packed cell units containing approximately 10^8 to 10^9 leukocytes per unit. A blood unit was transfused within 72 hours of donation. Serum samples were collected weekly from one to eight weeks after transfusion and then every three months until renal transplantation. Lymphocytotoxic anti-HLA antibodies were detected using a highly selected panel of 40 lymphocytes containing all HLA class I and class II. The National Institutes of Health (NIH) lymphocytotoxicity technique was used with and without dithiothreitol to separate IgM antibodies from IgG. Furthermore, blood samples from selected patients who were transfused were collected before transfusion and at 7, 28, and 60 days after transfusion for immunologic studies as described elsewhere [11, 12]. Blood samples from the blood donors were collected three months after the donation.

Tissue typing

Patients and blood donors were serologically typed for HLA antigens A and B by standard complement-dependent cytotoxicity assays. HLA DRB1 alleles were determined by genomic analysis: restriction fragment length polymorphism (RFLP) and/or polymerase chain reaction (PCR) using HLA sequence-specific oligonucleotides for DR loci (18 antigens at the generic level). A transfusion was considered HLA-DR matched if a minimum of one HLA-DRB1 allele was present in the transfusion donor as well as recipient. Forty-eight patients received a one DR-matched transfusion, and one received a two DR-matched transfusion. A transfusion was HLA-DR mismatched if no donor DRB1 allele was shared with the recipient. Of note, the mean HLA class I match per patient was similar in the two groups: 0.84 in the DR matched group and 1.1 in the DR mismatched group (difference P = NS).

Patients

We recruited a total of 144 patients who were randomized from June 1992 to January 1996, and 106 patients

Table 1. Summary of inclusions

	No transfusion	1 HLA-DR-matched transfusion	HLA-DR-mismatched transfusion	Total
Total included	47	49	48	144
Excluded	4	8	3	15
Died on waiting list	1	2	2	5
Transplanted	36	31	39	106
Still awaiting a graft	6	8	4	18

received a kidney transplant before the end of 1998 (Table 1). Forty-seven patients were allocated to remain untransfused. Forty-nine were to receive a one HLA-DR–matched transfusion, and 48 were to receive a one HLA-DR–mismatched transfusion. Twenty-five patients were withdrawn for the following reasons: five patients received additional transfusions (two in the nontransfused group, and three in the group receiving HLA-DR–compatible blood). Five patients died on the waiting list. Three patients refused transfusions after randomization to transfusion groups (two in the HLA-DR matched group and one in the HLA-DR mismatched group) for personal reasons. In one patient randomized in the transfused group, no HLA-DR–compatible donor was found. Two patients moved to another city and were withdrawn for logistical reasons. One patient was withdrawn from the waiting list for medical reasons. Two patients received a living donor graft and one a combined kidney-liver transplantation. Five patients died of complications during the dialysis period. Finally, 18 patients were still on the transplant waiting list at the end of the study. Patients who were transplanted had a minimum clinical follow-up of one year (median follow-up of 55 months, range of 17 to 83).

Immunosuppressive therapy

All transplant recipients included in the GCIF study received the same immunosuppressive protocol consisting of CsA, prednisone, azathioprine, and prophylactic induction with antilymphocyte preparation. Induction therapy consisted of 1.25 to 1.7 mg/kg antithymocyte globulins (ATG Thymoglobulin™; Imtix-Sangstat, Lyon, France) given for 10 days. CsA was started on day 9, at the initial daily dose of 6 to 8 mg/kg/day. Dosing was titrated to whole blood trough levels of 250 to 350 ng/mL for the period of days 9 to 42 and 150 to 250 ng/mL thereafter. Azathioprine administered at 1 to 2 mg/kg/day was started on day 1. A 2 mg/kg methylprednisolone bolus was given intravenously just before transplantation, and corticosteroids were tapered thereafter from 1 mg/kg at day 1 to 0.25 mg/kg at day 30 and to a daily fixed baseline of 10 mg from day 60. First-line antirejection therapy consisted of a daily 1 g intravenous methylprednisolone bolus over three days. ATGs, or muromonab-CD3 monoclonal antibody, were given as indicated in the case of refractory or rebound rejection if required. No other immunosuppressive drug was permitted during the six-month post-transplant period.

Study assessments and statistical analysis

The primary end points of the study were the number and the severity of first biopsy-confirmed acute rejections (Banff 93; borderline changes and grades I, II, and III) and the graft survival rates. Differences in categorical variables between the two groups were evaluated by chi-square analysis. Continuous variables were compared between the groups by two-tailed unpaired *t* tests. Survival rates and rejection cumulative incidence rates were computed according to the Kaplan–Meier method and are indicated as mean percentage ± SEM. It was estimated that a total of 87 transplant patients (29 in each group) would be required to detect a 30% significant difference in survival rates or rejection incidence with a power of at least 80%. The influence of factors on survival and rejection incidence was examined using the Cox proportional hazard model. Continuous variables were categorized in groups with approximately similar frequency except for age of donor (2 categories <50 years, ≥ 50 years) and cold ischemia time (<30 hours, ≥ 30 hours). To identify independent prognostic factors, multivariate stepwise regression analysis was performed using all of the factors included in the previous analysis. The proportionality assumption for each variable retained in the final model was verified before including them in the model. As the estimated hazard rate for graft survival and for first episode of rejection over time was not constant between the three randomization groups, this covariate was included in the model as a time-dependent covariate. Relative risks were presented as mean estimates with a 95% confidence interval (95% CI). In the statistical procedures, the threshold level for statistical significance was fixed at $P < 0.05$. The Statistical Package for Social Scientists (SPSS) was used for data management and analyses.

RESULTS

Study population

A total of 106 patients was included and received a transplant. Of these, 36 who were randomized in the nontransfusion group remained naive. Thirty-one were transfused with one unit of DR-matched blood, and 39 received one unit of DR-mismatched blood. The baseline characteristics of the three groups were similar with respect to age, sex, donor's age, and the HLA matching between the organ donor and the recipient (Table 2). The large majority of the study population was comprised of male dialysis patients. Patients were slightly but significantly ($P = 0.03$) older in the group of patients who

Table 2. Clinical characteristics of the study patients

Characteristics	No transfusion $N = 36$	1 HLA-DR-matched transfusion $N = 31$	HLA-DR-mismatched transfusion $N = 39$	P
Age *years*	34 ± 16	43 ± 12	39 ± 15	0.03
Sex *N*				
Male	29	29	36	NS
Female	7	2	3	
Lymphocytotoxic antibodies (IgG anti-T cells)	None	1 patient (1/20)	None	NS
Donor creatinine *μmol/L*	89 ± 38	96 ± 30	119 ± 68	0.02
Donor age *years*	37 ± 16	41 ± 12	39 ± 15	NS
Cold ischemia time *hour*	25 ± 8	27 ± 10	26 ± 8	NS
Patients with delayed graft function *N*	10 (28%)	9 (29%)	10 (26%)	NS
Mean HLA matching between kidney donor and recipient				
HLA-A identities	0.6 ± 0.6	1 ± 0.7	1.1 ± 0.5	0.03
HLA-B identities	0.7 ± 0.7	0.6 ± 0.6	0.7 ± 0.6	NS
HLA-DR identities	1.2 ± 0.7	1 ± 0.7	1.1 ± 0.5	NS
HLA-DR match between blood donor and kidney donor *N*				
0 match		15	29	
1 match		15	7	0.03
2 match		1	1	
Mean (range) time transfusion-transplantation *months*		18 ± 15 (3–62)	20 ± 16 (2–55)	NS
Post-transplant follow-up *months*	50 ± 23	48 ± 23	54 ± 24	NS

Plus–minus values are means ± SD.

received a DR-matched transfusion. Results of anti-lymphocytotoxic antibody detection after transfusion have been previously published in detail [13]. No patient developed anti–B-cell panel-reactive antibodies. Only one patient transfused with one DR-matched unit developed significant IgG anti–T-cell antibodies (1 out of 20, 5%), which were detected on day 35 and day 90 post-transfusion, respectively. The donor serum creatinine before procurement was significantly ($P = 0.02$) higher in the DR-mismatched transfused group. HLA-A matching between kidney donor and recipient was significantly ($P = 0.03$) better in the DR-mismatched transfused group compared with the nontransfused group. The incidence of delayed graft function, defined by the requirement of one post-transplant dialysis session or more within the first week post-transplant, was similar in the three groups. The mean time from transfusion to transplantation was also similar for patients of the two transfusion groups, and the mean post-transplant follow-up time was identical in the three groups.

Patient and graft survival

In total, six patients died during follow-up after transplantation: two in the nontransfused group, three in the one DR-matched transfused group, and one in the DR-mismatched transfused group. The causes of death were sepsis ($N = 2$), pneumonia ($N = 1$), liver failure ($N = 1$), adenocarcinoma ($N = 1$), and neurological complications of an inherited disease ($N = 1$). The patient survival rates (Kaplan–Meier method) for the study groups were not statistically significantly different between the three groups: 100%, 100%, and 97 ± 5% in the nontransfused group, the one DR-matched transfused group, and the DR-mismatched transfused group at one-year, respectively. At five years, patient survival rates were 92 ± 5.4%, 86 ± 6%, and 97 ± 5%, respectively. The predominant reasons for graft loss were chronic rejection, death, and refractory acute rejection (Table 3). The graft survival rates after transplantation (Kaplan–Meier method) were similar in the three groups at one-year (Fig. 1): 91.7 ± 4.6%, 90.3 ± 5.3%, and 92.3 ± 4.3%, respectively. At five years, the corresponding rates were 80 ± 6.8%, 79.3 ± 7.6%, and 83.7 ± 6.1%. In the Cox regression analysis, the factor of group randomization did not contribute significantly toward graft survival ($P = 0.41$) and the hazards ratio (HR) for nontransfused patients was 1.1 (95% CI, 0.51 to 2.48; $P = 0.76$) and for patients transfused with DR-matched blood was 0.59 (95% CI 0.22 to 1.55; $P = 0.28$). The occurrence of rejection was the only risk factor that contributed significantly ($P = 0.05$) to graft loss (HR for graft loss, 2.92; 1.01 to 8.43,

Table 3. Reasons for graft loss

	No transfusion $N = 36$		1 HLA-DR-matched transfusion $N = 31$		HLA-DR-mismatched transfusion $N = 39$	
	N	%	*N*	%	*N*	%
Death	2	5.6	3	9.7	1	2.6
Refractory acute rejection	2	5.6	1	3.2	—	—
Chronic rejection	2	5.6	1	3.2	3	7.7
Primary non-function	1	2.8	—	—	—	—
PTLD	—	—	—	—	1	2.6
Other	—	—	1	3.2	1	2.6
Total	7	19.4	6	19.3	6	15.4

PTLD is Post-transplant lymphoproliferative disease.

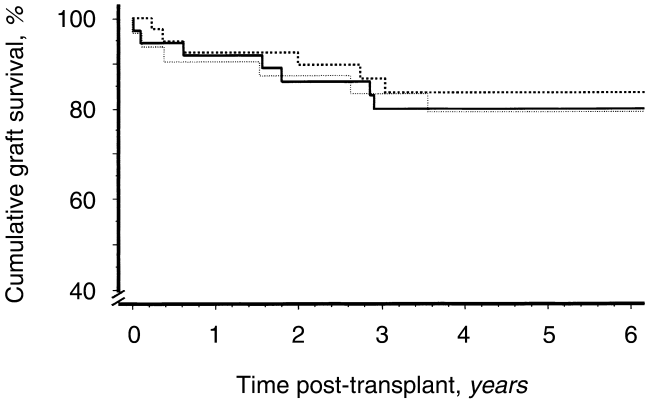

Fig. 1. Five-year kidney graft survival in patients who received a single pre-transplantation blood unit, with matching for one human lymphocyte antigen (HLA)-DR or without HLA-DR matching between recipients and blood donors, and in patients who received no transfusion. Symbols are: (——) non-transfused patients ($N = 36$); (– – –) one DR-matched unit transfused patients ($N = 31$); (••••) DR-mismatched unit transfused patients ($N = 39$).

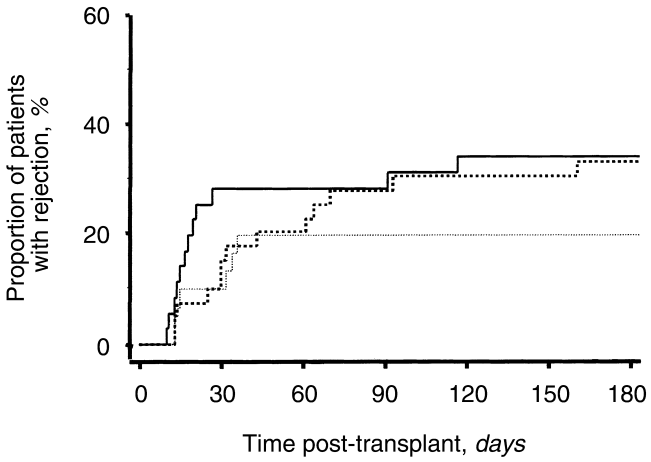

Fig. 2. Kaplan–Meier estimates of the occurrence of first rejection during the six-month post-transplant period for the three groups. Symbols are: (——) non-transfused patients ($N = 36$); (– – –) one DR-matched unit transfused patients ($N = 31$); (••••) DR-mismatched unit transfused patients ($N = 39$).

95% CI). There was no significant contribution of the other risk factors: age of recipient; HLA-A, HLA-B, and HLA-DR matching with the kidney donor; donor age; sex and serum creatinine; and occurrence of delayed graft function.

Rejection incidence and severity

We analyzed the incidence of biopsy-confirmed acute rejection episodes during the six-month post-transplant period. No patient received antirejection therapy without transplant biopsy being performed before or within 48 hours after antirejection therapy initiation. During this period, acute rejection (defined as borderline changes or a grade of 1 or higher according to the Banff 1993 criteria) developed and required antirejection therapy in 12 of the 36 patients in the nontransfused group (33.3%) as compared with 6 of the 31 in the one DR-matched transfused group (19.4%), and 13 of the 39 in the DR-mismatched transfused group (33.3%). The tendency to observe a lower frequency of rejection observed in the one DR-matched transfused group was not statistically significant (RR 0.59; 95% CI, 0.22 to 1.55; $P = 0.28$). The fraction of patients who remained free of rejection during the first six months was similar between the three groups (Fig. 2). In the multivariate Cox regression analysis, neither DR matching with the kidney donor (RR for 0 DR match 1.5; 95% CI, 0.42 to 5.24; $P = 0.54$) nor the age of recipient and the occurrence of post-transplant acute tubular necrosis contributed significantly toward acute rejection. After six months, only three other patients had experienced late acute rejection: one patient in the one DR-matched transfused group at day 341 and two in the DR-mismatched transfused group at days 520 and 868, respectively. The median onset of first rejection tended to be observed later in the group of patients transfused with DR-mismatched blood (mean ± SEM): 32 days (50 ± 12) versus 23 days (24 ± 5) in the group of patients transfused with one DR-matched unit and 17 days (mean ± SEM, 31 ± 10) in the nontransfused group, but this trend was not statistically significant.

Overall, the severity of rejection was comparable within the three groups (Table 4). A similar number of patients had multiple episodes of rejection, and a similar number of patients required rescue therapy with muromonab-CD3 or antithymocyte globulin for the treatment of rejection. Refractory rejection justified a switch from CsA to tacrolimus (0.1 mg per kg and per day) in five patients, including three patients transfused with DR-mismatched blood, one patient transfused with DR-compatible blood, and one nontransfused patient. Eight rejection episodes (44% of rejections) of grade 2 or grade 3, in which vascular changes were present in transplant biopsy, occurred in the group of patients transfused with DR-mismatched blood. This proportion was slightly but nonsignificantly lower in the group transfused with one DR-matched blood and the nontransfused group: 30 and 25% of rejection episodes, respectively.

DISCUSSION

The transfusion effect in kidney transplantation, established by most clinical and experimental studies in the 1970 to 1980s, was later questioned in the light of the risk of transmitting infectious disease and of the detrimental effect of sensitization, especially when protocols included several random transfusions. However, an amazing theory reappraised the transfusion story when it was suggested by retrospective clinical data that HLA matching

Table 4. Frequency and severity of rejection episodes during the six-month post-transplant period

Variable	No transfusion $N = 36$	1 DR-matched transfusion $N = 31$	DR-mismatched transfusion $N = 39$
Number of patients with acute rejection	12 (33.3%)	6 (19.4%)	13 (33.3%)
Total number of acute rejection episodes	16	10	18
Frequency of rejections *episodes/patient*	0.44	0.32	0.46
Number of patients with recurrent or resistant rejections	4 (11.1%)	3 (9.7%)	5 (12.8%)
ALG/ATG/OTK3 use			
Number of patients	4 (11.1%)	3 (9.7%)	3 (7.7%)
Histology			
Number of Grade 1–Borderline rejections	12 (75%)	7 (70%)	10 (56%)
Number of Grade 2–Grade 3 rejections	4 (25%)	3 (30%)	8 (44%)

Abbreviations are: ALG, antilymphocyte globulin; ATG, antithymocyte globulin; OKT3®, muromonab-CD3.

between the transfusion donor and the recipient might have been the crucial factor influencing the "tolerogeneic" effect of a single blood transfusion [14]. Indeed, sharing one HLA haplotype or at least one HLA-DR antigen was reported to improve graft survival or to reduce the incidence of post-transplant acute rejection episodes significantly in kidney graft recipients [6]. Importantly, experimental data, in conjunction with clinical studies, showed that transfusions might have different in vitro immunomodulatory effects, according to the degree of HLA antigen sharing between the transfusion donor and the recipient. Sharing of one haplotype, or at least one DR and one B antigen, led to deletion and/or inactivation of donor-specific cytotoxic T-cell precursors (CTLp) that are associated with a selective loss of T-cell receptor Vβ gene expression [15]. In studies conducted in transplant patients, low frequencies of donor-specific cytotoxic T-lymphocyte precursors correlated with favorable graft outcome, emphasizing the clinical relevance of a specific decrease in T-cell responses against alloantigens. Therefore, some authors postulated that a tolerance state could be induced by low-grade mixed chimerism with donor lymphocytes homing in privileged sites after blood transfusion [16], and that the induction of such chimerism might depend on the degree of HLA matching between the transfusion donor and the recipient. Other authors have reported that microchimerism was detectable up to eight weeks after transfusion and tended to be of shorter duration after mismatched transfusions [17]. In contrast, patients who received a mismatched transfusion maintained or increased donor-specific CTLp frequencies and developed high-avidity CD8-independent CTL clones, which might be hazardous for the subsequent allograft [18]. However, further in vitro studies, including ours, were unable to confirm the results of van Twuyver et al [11, 19–21]. Various explanations have been proposed to explain these discrepancies, such as the low number of leukocytes transfused in some studies [21] or the modulatory effect of transfusion on interleukin-2–producing helper T lymphocytes [20]. Another explanation may be the use of different techniques: by adding exogenous interleukin-2 to the culture medium in order to ensure T-cell expansion, we were more easily able to detect donor-specific sensitization of CTL after transfusion, and the rate, kinetics, and intensities of such sensitization were not significantly different between recipients of a one HLA-DR–matched transfusion and of a mismatched transfusion [12]. Furthermore, we were unable to detect any significant microchimerism, as only transient microchimerism was observed at one and/or four weeks after transfusion in 4 out of 22 patients transfused with one DR-antigen–matched blood and 4 out of 19 patients transfused with mismatched blood (unpublished personal data). Recent data from Strober et al, in a cadaver renal transplant patient given pretransplant total lymphoid irradiation and withdrawn from immunosuppressive drugs more that 12 years ago, showed that tolerance was maintained in the absence of detectable donor microchimerism and in the presence of a vigorous reactivity to donor stimulator cells in the mixed leukocyte reaction, suggesting that neither chimerism nor clonal deletion or anergy is required [22]. Our clinical data confirm these experimental results. In the present prospective, randomized trial, patient survival, kidney graft survival, and the incidence of acute biopsy-confirmed rejection did not differ significantly between the three study groups defined according to the pre-transplant transfusion policy: one HLA-DR match transfusion, one HLA-DR–mismatch transfusion, or no transfusion. In other words, in our study, the history of transfusion was not a significant risk factor for graft outcome parameters. Lagaaij et al reported a significantly better five-year graft survival in renal transplant patients who received a transfusion matched for one HLA-DR antigen: 81 versus 57% in those who received blood not matched and 45% in those who remained naive untransfused [6]. Such results, however, refer to a historical period when recipients were treated with azathioprine and steroids, and did not benefit from the huge improvement in transplantation results observed with newer immunosuppressants. At that time, figures of 90% one-year graft

survival and 20% post-transplant rejection incidence were commonly recorded. Indeed, further retrospective studies and prospective trials on HLA-matched transfusion were unable to find any influence of transfusion history on kidney graft survival. van Hoof and van den Berg-Loonen failed to confirm the influence of DR matching between the blood donor and the recipient in 147 naive dialysis patients, including 38 who were transplanted with CsA-based immunosuppression [9]. Middleton et al compared sensitization and graft outcome of transplant patients who had been given one-HLA-DR antigen-matched blood transfusion before transplantation with control patients who received blood from random donors [23]. No difference in sensitization or in graft survival was found. The incidence of rejection episodes was significantly reduced, but for comparison the investigators used a historical control group who had received random transfusions for which the match grade of the donors was unknown. The most recent study on this topic, published by Christiaans et al, was also retrospective and used leukocyte-poor erythrocytes [10]. They did not find a significant difference in antibody formation after transfusion, in rejection rate, or in graft survival after transplantation between patients receiving DR-matched and DR-mismatched blood.

Bayle et al used a different approach by analyzing the effect of a single haplo- or semi-identical blood transfusion in 52 kidney transplant recipients compared with a historical group of 53 transplant patients in which three random transfusions were given [8]. They found a very significant beneficial effect of haploidentical transfusions performed in naive patients. Naive patients from this group did not develop post-transfusion HLA antibodies, and they experienced significantly fewer episodes of acute rejection (2.7% of 36 patients) compared with 48 naive control patients (20.8%), but no statistical difference was found in the whole population whatever the type of transfusion used. Blood donors were blood- and bone marrow-registered donors as well as the patients' family, but no separate analysis was done according to donor type. Another similar study was unable to confirm these preliminary results [24].

While limited by the small size of the sample, our study was properly designed, including a homogeneous cohort of selected naive patients—that is, untransfused, without previous pregnancy or previous transplant—and it was the first to our knowledge that was randomized with an untransfused control group. Our trial was designed in 1992, and considering the data of Lagaaij et al, we speculated that the five-year survival would improve by 35% and/or the graft rejection episode incidence be reduced by 30% with DR-matched transfusions [6]. In all patients who were transplanted, we used a quadruple immunosuppressive combination with CsA, ATG, azathioprine, and steroids, which was the most powerful drug treatment available before the approval of newer agents such as tacrolimus, mycophenolate mofetil, sirolimus, and anti-CD25 monoclonal antibodies. We cannot exclude, however, that transfusions might have different immunologic modulatory effects according to the HLA compatibility between donor and recipient. These effects are only observed in vitro after transfusion and are weak and transient, and their possible influence on the graft outcome remains questionable. Considering the costs and the considerable logistical efforts required to select and type blood donors and despite the low risk of deleterious sensitization (5% in the present study), a routine policy of HLA-compatible transfusion appears both prohibitive and expensive and, therefore, is impractical in most transplant centers.

In large transplant registries, such as the UNOS and Collaborative Transplant Study databases, graft survival in nontransfused patients was reported in 1987 to 1988 to become the same as for transfused recipients. Accordingly, the transfusion policy has changed to liberal use or strict avoidance in a majority of worldwide kidney transplant centers, but some individual centers continue to routinely apply a deliberate transfusion policy. In children, known to mount severe immunologic reactions after kidney transplantation, a retrospective study was recently published showing that in patients who received two transfusions under CsA coverage, no sensitization occurred, and graft survival rates were significantly improved [25]. However, the authors had mixed recipients of cadaver donor grafts who received random transfusions with recipients of living donor grafts in which donor-specific transfusions were given. On the other hand, survival rates were compared with a historical group of patients who were transplanted during the previous period.

Of importance, the results of the Prospective International Collaborative Study on pretransplant transfusions coordinated by Opelz, conducted from 1987 to 1994, have established that graft survival was significantly higher in 205 transplant patients who received three random blood transfusions prior to transplant than in the 218 untransfused patients (at 1 year, 90 vs. 82%, $P = 0.02$; at 5 years, 79 vs. 70%, $P = 0.025$) [26]. Immunosuppression protocols included CsA/prednisone or triple-drug regimen with CsA, prednisone, and azathioprine. The proportion of patients who remained free of rejection was similar in the transfusion (46%) and the transfusion-free (45%) groups. Because repeat rejections were more frequent in the untransfused group, the authors assumed that the severity of rejections was more pronounced when transfusions were not given. However, in this study, the baseline characteristics of the patients were not given, and a bias in patient selection cannot be excluded. Furthermore, the causes of graft failures are not mentioned, and it cannot be excluded that technical or surgical failures, not related to immunologic events,

might have been responsible for the lower graft survival in the group of untransfused patients. Finally, with the availability of newer immunosuppressive agents, it has been demonstrated that excellent results can be achieved with a low (10 to 20%) incidence of rejection episodes. Interestingly, during the same period as when the transfusion effect was disappearing, UCLA/UNOS data showed that there was a gradual reduction in the effect of immunologic risk factors previously known to determine the survival of transplants, such as sensitization and HLA matching, while other demographic factors such as the age of donors and the age of recipients have remained almost unchanged [27].

In summary, in our opinion, the debate on the interest of routine blood transfusions in kidney transplantation is to a large extent obsolete at the dawn of the third millennium. Attention should focus on factors having an impact on the quality of transplant kidneys (warm and cold ischemia times, damage occurring during procurement) and factors able to compromise the renal mass of the kidney: age and sex of the donor, as well as the proper use of nephrotoxic anticalcineurine agents, which are becoming the key factors influencing the long-term function of renal transplants. The intrinsic risk of transfusion is definitely minimal, and our study is not sufficiently powerful to exclude a small immunomodulatory effect; however, a chance of deleterious anti-HLA immunization remains, which may close the transplantation gateway to an individual patient, particularly given the scarcity of organ supply for subjects with the uncommon ABO and/or HLA groups. It may be more cost-effective to avoid transfusion, particularly in high-risk patients, rather than to continue hazardous transplantation attempts in highly sensitized recipients, after removal of anti-HLA antibodies by plasmapheresis or immunoadsorption.

ACKNOWLEDGMENTS

This work was supported by the "Délégation à la Recherche Clinique de l'Assistance Publique des Hôpitaux de Paris" (Grants 920604 and 930615) and by the "Programme Hospitalier de Recherche Clinique" (Grant AOA94043). This study was presented in abstract form at the 18th International Congress of the Transplantation Society, Rome, Italy, 2000. The authors also acknowledge Dr. Michel Bouvier d'Yvoire and Mr. Nigel Strang, Etablissement Français des Greffes, who carefully reviewed the manuscript.

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REFERENCES

1. Opelz G, Sengar DPS, Mickey MR, Terasaki PI: Effect of blood transfusions on subsequent kidney transplants. *Transplant Proc* 5: 253–259, 1973
2. Van Es AA, Balner H: Effect of pretransplant transfusions on kidney allograft survival. *Transplant Proc* 11:127–131, 1979
3. Time to abandon pre-transplant blood transfusions? (editorial) *Lancet* 1:567–568, 1988
4. Kerman RH, Van Buren CT, Lewis RM, Kahan BD: Successful transplantation of 100 untransfused cyclosporine-treated primary recipients of cadaveric renal allografts. *Transplantation* 45:37–40, 1988
5. Hiesse C, Lang P: Transfusion protocols prior to renal transplantation: Questionnaire on current attitudes at French centers. *Transplant Proc* 27:2180–2181, 1995
6. Lagaaij EL, Hennemann PH, Ruigrok M, *et al:* Effect of one-HLA-DR-antigen-matched and completely HLA-DR-mismatched blood transfusions on survival of heart and kidney allografts. *N Engl J Med* 321:701–705, 1989
7. Middleton D, Martin J, Douglas JF: Effect on sensitization of giving HLA-DR-matched blood to potential renal transplant recipients. *Transplant Proc* 24:2535–2536, 1992
8. Bayle F, Masson D, Zaoui P, *et al:* Beneficial effect of one HLA haplo- or semi-identical transfusion versus three untyped blood units on alloimmunization and acute rejection episodes in first renal allograft recipients. *Transplantation* 59:719–723, 1995
9. van Hoof JP, Van Den Berg-Loonen PM: The influence of DR match of blood donor and recipient on the formation of T- and B-cell antibodies and on renal allograft outcome. *Transplant Int* 5(Suppl 1):S599–S600, 1992
10. Christiaans MHL, Van Hoof JP, Nieman F, Van Den Berg-Loonen EM: HLA-DR matched transfusions. *Transplantation* 67:1029–1035, 1999
11. Baudouin V, Ansart-Pirenne H, Soulimani N, *et al:* Cytokine mRNA and protein expression in a mixed leukocyte reaction before and after allogeneic transfusions. *Transplantation* 66:376–384, 1998
12. Baudouin V, De Vitry N, Hiesse C, *et al:* Cytotoxic changes after HLA-DR match and HLA-DR mismatch blood transfusions. *Transplantation* 63:1155–1160, 1997
13. Busson M, Hiesse C, Buisson C, *et al:* Lymphocytotoxic antibody responses to one prospective HLA-DR typed blood transfusion in naive patients awaiting renal transplantation. *Transplant Proc* 29:1415–1416, 1997
14. Sachs DH: Specific transplantation tolerance. *N Engl J Med* 325: 1240–1242, 1991
15. van Twuyver E, Mooijaart RJD, Ten Berge IJM, *et al:* Pretransplant blood transfusions revisited. *N Engl J Med* 325:1210–1213, 1991
16. De Waal LP, Van Twuyver E: Blood transfusions and allograft survival: Is mixed chimerism the solution for tolerance induction in clinical transplantation? *Crit Rev Immunol* 10:417–425, 1991
17. Vervoordeldonk SF, Doumaid K, Remmerswaal EB, *et al:* Long-term detection of microchimaerism in peripheral blood after pretransplantation blood transfusion. *Br J Haematol* 102:1004–1009, 1998
18. van Twuyver E, Mooijaart RJD, Ten Berge IJM, *et al:* High-affinity cytotoxic T lymphocytes after non-HLA-sharing blood transfusion: The other side of the coin. *Transplantation* 57:1246–1251, 1994
19. van Der Mast BJ, Viëtor HE, Van Der Meer-Prins EMW, *et al:* Modulation of the T cell compartment by blood transfusion. *Transplantation* 63:1145–1154, 1997
20. Young NT, Roelen DL, Iggo N, *et al:* Effect of one-HLA-haplotype matched and HLA-mismatched blood transfusions on recipient T lymphocyte allorepertoires. *Transplantation* 63:1160–1165, 1997
21. van Prooijen HC, Aarts-Riemens MI, Van Oostendorp WR, *et al:* Prevention of donor-specific T-cell unresponsiveness after buffy-coat-depleted blood transfusion. *Br J Haematol* 19:219–225, 1995
22. Strober S, Benike C, Krishnaswamy S, *et al:* Clinical transplantation tolerance twelve years after prospective withdrawal of immunosuppressive drugs: Studies of chimerism and anti-donor reactivity. *Transplantation* 69:1549–1554, 2000
23. Middleton D, Martin J, Douglas J, McClelland M: Transfusion of one HLA-DR antigen-matched blood to potential recipients of a renal allograft. *Transplantation* 58:845–848, 1994
24. Mariat C, Alamartine E, De Filippis JF, *et al:* Effect of HLA

semi-identical pretransplant blood transfusions on renal allograft outcome. *Transplant Proc* 32:381–383, 2000
25. Niaudet P, Dudley J, Charbit M, *et al:* Pretransplant blood transfusions with cyclosporine in pediatric transplantation. *Pediatr Nephrol* 14:45–51, 2000
26. Opelz G, Vanrenterghem Y, Kirste G, *et al:* Prospective evaluation of pretransplant blood transfusions in cadaver kidney recipients. *Transplantation* 63:964–967, 1997
27. Yuge J, Terasaki PI: The transfusion effect relative to other graft survival factors, in *Transplantation and Clinical Immunology* (vol XXIV), edited by Touraine JL, Traeger J, Amsterdam, Elsevier Science, 1992, pp 49–53