Transplant Int (1991) 4: 235–238

TRANSPLANT
International

Effect of the number of pregraft blood transfusions in kidney graft recipients treated with bioreagents and cyclosporin A

R. Baatard[1], J. Dantal[1], M. Hourmant[1], D. Cantarovich[1], A. Cesbron[2], J. D. Bignon[2], and J. P. Soulillou[1, 3]

[1] Service de Néphrologie et d'Immunologie Clinique, Centre Hospitalo Universitaire, 1, Place Alexis Ricordeau,
[2] Centre de Transfusion Sanguine de Nantes, and [3] Unité INSERM 211 "Unité de Recherche sur les Effecteurs Lymphocytaires T", Faculté de Médecine, F-44035 Nantes, France

Received December 27, 1990/Received after revision June 26, 1991/Accepted July 1, 1991

Abstract. The impact of a systematic, nondonor-specific, pregraft blood transfusion (BT) protocol was evaluated retrospectively in 446 consecutive, first renal transplant recipients with regard to graft survival rate, rejection, and incidence of infectious episodes. Cyclosporin A was the maintenance immunosuppressive treatment in all patients after a 2-week course of antithymocyte globulin or anti-IL-2 monoclonal antibody. Recipients were assigned to three groups according to the number of pregraft BT (one or two, three or four, or more than four). When nonimmunological failures were excluded from the study, patients receiving three or four BT had statistically better graft survival ($P < 0.02$) and a lower incidence of rejection episodes ($P < 0.05$) than those in the other groups. There were no significant differences between the three groups in the distribution of HLA mismatching (A, B and DR), time interval between the last BT and transplantation, DR6 recipient phenotype, or nonimmunological failures. Our results show that the number of pregraft BT is an important factor in transplantation.

Key words: Kidney transplantation, blood transfusion – Blood transfusion, kidney graft survival

The beneficial effect of pregraft blood transfusions (BT) has been well documented in animals [5, 8, 16, 24] and humans [9, 10, 21], and systematic BT policies are now the rule prior to renal transplantation. Today, as new immunosuppressive regimens, including bioreagents and cyclosporin A (CyA), increase the rate of successful graft function, the beneficial effect of BT must be reappraised. Some recent reports advocate BT [10, 17, 21], but similar graft survival rates in nontransfused, CyA-treated patients have also been published [4, 13, 15]. These conflicting data are all the more reason to reappraise pregraft BT benefit while taking into consideration the increasing risk of viral contamination (e.g., from human immunodeficiency virus, hepatitis or cytomegalovirus) or of HLA immunization. Optimal BT parameters (number, timing, quality, etc.) need to be determined in transfused populations. As a result of increasing rates of actuarial graft survival, more detailed analyses of rejection frequency [14], level of graft function, and incidence of infectious episodes are also required.

This paper reports the results of a retrospective study of BT protocol in an ABO isogroup of kidney allograft recipients treated with a stereotypic immunosuppressive regimen including a bioreagent and CyA. Optimal results were achieved when a moderate number of BT – three or four – were performed before grafting.

Materials and methods

Patients and transfusion protocols

Only consecutive, first cadaveric graft recipients (287 males, 159 females; mean age 39.3 years, range 6–66 years) were included in the study. Follow-up was more than 1 year for 319 patients and more than 4 years for 103. All patients were prospectively and deliberately transfused prior to transplantation according to a protocol described in detail elsewhere [23]. Briefly, patients received one BT (one packed 250-ml blood unit) every 3 months starting from the time they were placed on a waiting list. Panel-reactive antibodies (PRA) against T and B lymphocytes were checked serially after BT (days 15 and 21) and every 2 months. This systematic BT regimen was discontinued if patients had immunization above 20% of anti-T PRA or 40% of anti-B PRA, as assessed by lymphotoxicity on a panel of 30 cells. Some patients received one or more additional BT units in case of emergency or of leukocyte-poor blood resulting from chronic anemia. Finally, all patients were systematically transfused (two units) during transplant surgery.

In order to assess the minimum number of BT that would produce the most beneficial effect, patients were divided into three groups, depending on the number of pregraft BT received (one or two, three or four, or more than four). The distribution of the patients excluded from and included in the three groups was: 13/126 (103%), 15/122 (123%), and 23/198 (116%), respectively. The distribution of pregnancies (105 women) was not statistically different between groups. Within this follow-up group, 51 patients (11%) lost their graft due to technical or nonimmunological failure (including arterial and venous thrombosis, ureteral necrosis, accidental death,

Offprint requests to: J. P. Soulillou, Service de Néphrologie et d'Immunologie Clinique, Centre Hospitalo Universitaire, 1, Place Alexis Ricordeau, F-44035 Nantes, France

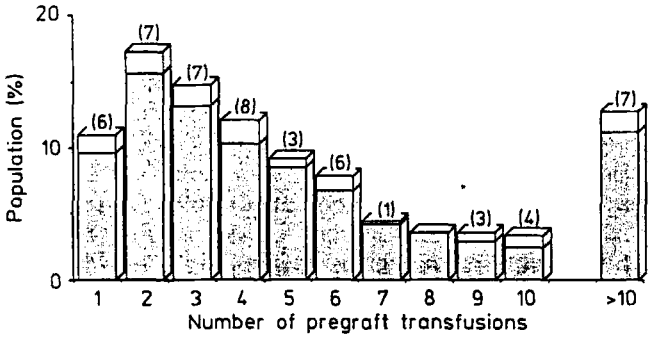

Fig. 1. Histogram of the number of pregraft blood transfusions (BT) in the population studied. BT given systematically on the day of transplantation are not included. ■ Patients included in the groups; □ patients excluded due to nonimmunological failure of graft

and cardiovascular stroke). There were no statistical differences between groups with regard to these incidents. Data are given with the incidents excluded and included since a specific BT effect may have been involved.

The mean mismatching indices were 0.98 ± 0.6 and 1.12 ± 0.6, respectively, for HLA-DR-B and -A antigens. All transplantations were performed with a negative crossmatch on donor T lymphocytes using current sera, regardless of reactivity on donor B lymphocytes, as described by Bignon et al. [1].

Immunosuppressive regimen

During the first 2 weeks after transplantation, patients were treated using a sequential prophylactic strategy including either antithymocyte globulin (ATG, Institut Mérieux, Lyon, France; 78%), antiblast globulin [3, 11] (3%), or an anti-IL-2 receptor monoclonal antibody [25, 26] (19%), followed by CyA as previously described [26]. Between January 1982 and April 1988, prednisone (Pred) and azathioprine (Aza) were definitively stopped after day 45. After April 1988, the maintenance regimen consisted of a combination of CyA and Aza, as described previously [12], and only Pred was stopped on day 45. Patients with chronic rejection or chronic CyA toxicity received triple therapy (CyA, Aza, and Pred). Acute rejection episodes were treated with boluses of Pred for 5 consecutive days (cumulative bolus dose 20 mg/kg), followed by 1 mg/kg oral Pred, gradually tapered (10 mg/week) until definitive withdrawal.

Statistical analysis

Graft survival, expressed as actuarial survival, was compared using the log-rank test. Differences in distribution percentages were compared using the nonparametric Kruskal-Wallis analysis when the chi-square test could not be applied to ordinal data.

Results

Number of transfusions and immunization

The number of BT before transplantation is given in Fig. 1: 28.2% of the patients received one or two BT, 26.9% three or four, and 44.8% more than four. These groups were arbitrarily defined to represent the usual pregraft transfusion categories and to facilitate statistical analysis by obtaining relatively balanced groups. At the time of transplantation, 65% of the patients had no anti-T PRA, 25% had 4%–50%, 4% had 50%–80%, and 6% had more than 80%. The percentage of patients who became immunized before transplantation (i.e., > 4% PRA) was significantly ($P < 0.05$) and linearly ($r = 0.63$) correlated with the number of pregraft BT (Fig. 2). When the correlation is made according to level of immunization, only patients with less than 50% anti-T PRA are involved, suggesting that hyperimmunization cannot be predicted from the number of BT.

The distribution of blood groups was statistically significant ($P = 0.005$) within the three groups. Patients in blood group O were transfused more often than those in group A (7.57 BT vs 5.83; NS), probably because of their longer dialysis period ($P < 0.001$). There were more O (32.4%) than A (15.1%) patients in the group with intermediate immunization (4%–50% anti-T PRA; $P < 0.005$). Moreover, O patients were more closely matched with their donors (four HLA-B-DR mismatches: 16.55% A, 4.23% O; $P < 0.001$ and full HLA mismatches: 27.4% A, 6.5% O). Conversely, match distribution (HLA-A, B, DR and B-DR) was independent of the BT groups studied (data not shown).

Patient and graft survival; rejection incidence

Actuarial survival according to the number of BT is shown in Fig. 3 A (NS). Patients with three or four BT had better graft survival than those with one, two or more than four, although this difference was not statistically significant (Fig. 3 B). Technical and nonimmunological failures were the major cause of graft loss (51/450; 11%), and the incidence of these failures had no significance according to BT group. When these failures are excluded (Fig. 3 C), the differences become statistically significant, indicating that patients with three or four BT had better graft survival

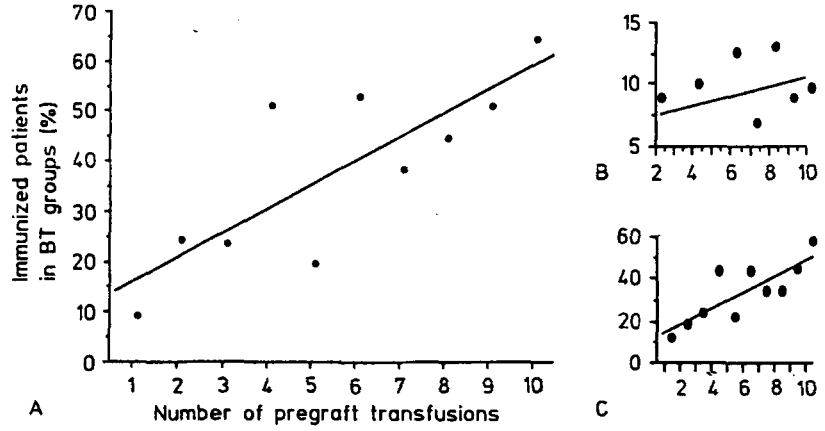

Fig. 2. A Correlation between the number of BT received before grafting and immunization against the panel of allogeneic T lymphocytes. Patients were considered as immunized when anti-PRA blood level was above 4%. The correlation is statistically significant (Kruskal-Wallis $P < 0.005$, $r = 0.63$). **B, C** When the transfused population is divided according to PRA level, the correlation is not significant in highly immunized patients (**B**: anti-T PRA > 50%, $r = 0.35$; **C**: anti-T PRA 0%–50%, $r = 0.82$)

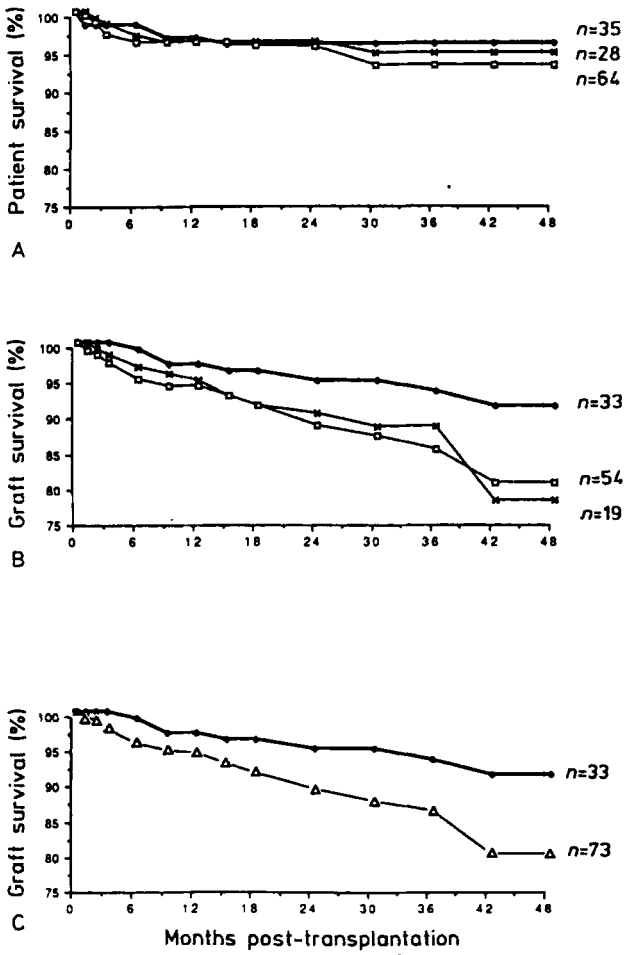

Fig. 3. A Actuarial patient survival in the three groups of patients studied. Differences are not statistically significant (log rank). x—x 1–2 BT; ●—● 3–4 BT; □—□ > 4 BT **B** Actuarial graft survival according to the number of BT received (NS: $X^2 = 1.4$ for 2 dF at 48 months). Symbols as for **A**. **C** Actuarial graft survival in the patients with three to four BT and in the other two groups combined. The differences are significant if nonimmunological failures ($n = 51$, 11% of all patients) are excluded ($P < 0.02$, $X^2 = 5.75$ for 1 dF at 48 months; $P = 0.05$, $X^2 = 3.98$ for 1 dF at 36 months). The number of patients at 48 months are indicated for each group. ●—● 3–4 BT; Δ—Δ < 3 and > 4 BT

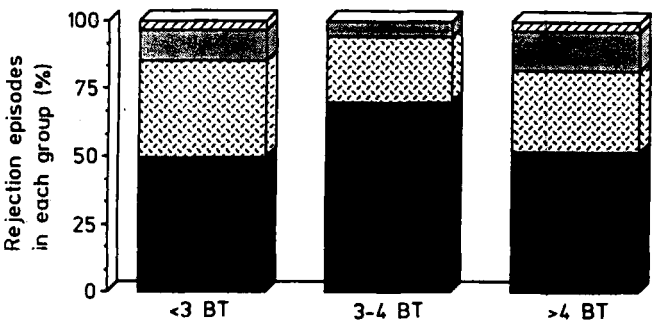

Fig. 4. Incidence of acute rejection episodes according to the number of BT received before transplantation. Incidences are statistically significant (Kruskal-Wallis, $P < 0.005$) among the groups. This difference is also significant when assessed group by group ($P < 0.05$ for group < 3 BT vs group 3–4 BT and for group > 4 BT vs group 3–4 BT). ■ No rejection; ▨ 1 rejection; ▩ 2 rejections; ▨ > 2 rejections

than those in the other groups taken together ($P < 0.02$ at 48 and 60 months). Moreover, patients transfused three or four times prior to transplantation also had a significantly lower graft rejection incidence than those who received one, two, or more than four BT ($P < 0.05$ in both cases; Fig. 4). HLA matching, duration of dialysis, and PRA levels were not statistically different in the groups studied and thus could not account for the variations observed.

Finally, the mean time interval between the last BT and transplantation did not differ in the three groups. There was no statistical difference in the time interval between the last BT (< 1, < 3, or < 6 months) and transplantation (data not shown), although graft outcome always ranked better when the BT was closest to transplantation time.

Infectious episodes

The incidence of overt cytomegalovirus disease episodes, herpes simplex, septicemia, and urinary tract infections was analyzed according to the number of BT received. There were no significant differences in the frequency of these infectious episodes. However, when analysis was performed according to the number of BT, assessed unit by unit, there was a significantly higher incidence of herpes simplex infection (33%) in patients who received more than eight BT than in others ($P > 0.05$). The time interval between BT and transplantation had no influence on incidence of infection.

Discussion

Before the development of new immunosuppressive regimens including bioreagents and CyA, it was assumed that BT had a beneficial effect on graft survival [17, 19, 21]. This assumption has recently been contested in reports showing no statistically significant difference in survival between nontransfused and transfused patients [4, 13, 15]. This apparent discrepancy could be related to differences in BT preparation protocol (i.e., packed blood versus frozen blood, etc.) or to practices in different centers. Our results in a population studied within a single center and receiving a uniform therapeutic regimen indicate that patients transfused three or four times prior to transplantation had the best graft survival. These same patients also had a lower incidence of acute rejection episodes than those with less than three or more than four BT. However, these effects were only statistically significant when nonimmunological failures were excluded. Such exclusion could be considered logical. BT effect is probably mediated by immunological mechanisms, and nonimmunological factors are the major cause of graft failure. Before the CyA era, other authors had suggested that 6–20 BT had a beneficial effect [18, 19]. In our study, this benefit was shown for a lower number of BT (i.e., three to four). In addition, graft outcome appeared to be better when the last transfusion was performed near the time of transplantation, although results were not statistically significant.

Although it has recently been reported that a beneficial transfusion effect is observed in patients without HLA-DR matching [6, 15, 22], the better results in our three to four BT group could not be attributed to a significant dif-

ference in HLA matching. Duration of dialysis, PRA levels, time interval between the last BT and transplantation, and recipient DR6 phenotype were not significantly different between our groups. Although there were more blood group A patients in the one to two BT group, this fact cannot account for the improved effect of three to four BT since there were no differences in graft outcome or rejection incidence according to A, B, and O distribution within the whole population or in the BT groups.

Our results suggest that the number of BT received prior to transplantation is an important factor in determining how beneficial the transfusion effect will be. This factor may be affected by the use of other blood preparations since the choice of an optimal number of BT is probably dependent on the type of blood used and may thus be valid only for a given clinical practice. Our experience indicates that no more than four BT are required and that larger quantities of blood may correlate with increased incidence of viral infection [7, 22], cancer [2], or risk of HLA immunization.

A recent multicenter study [20] indicated that graft outcome remained slightly better in transfused than in nontransfused patients, even with CyA as maintenance therapy. One-year actuarial graft survival was increased by 6% after a single transfusion (75%) but was not statistically improved by additional transfusions (79% for three to four BT). However, our study suggests that the effect of BT may be optimal in patients receiving three to four of them. Survival was 9.6% better at 42 and 48 months compared to all other patients. This would seem to justify continued application of a systematic BT policy, provided that careful screening for viral contamination is performed. This regimen should be limited to three to four BT since a greater number has no beneficial effect and increases the potential risk of HLA immunization.

References

1. Bignon JD, Le Forestier M, Hourmant M, Klaussmann G, Soulillou JP (1985) Prevalence in risk factors of hyperimmunization in patients awaiting grafts. Effect of grafting through a positive B-lymphocyte cross-match. In: Touraine JL, Traeger J (eds) Transplantation and clinical immunology, vol 17. Excerpta Medica, Amsterdam, pp 57–69
2. Blumberg N, Heal J, Murphy P (1986) Association between transfusion of whole blood and cancer recurrence. Br Med J 293: 530–532
3. Bonneville M, Carcagne J, Vié H, Moreau JF, Chevalier S, Latour M, Carosella E, Soulillou JP (1989) Polyclonal rabbit gammaglobulins against a human cytotoxic CD4+ T cell clone. I. Clone characteristics and antiblast globulin (GAB) preparation. Transplantation 48: 253–260
4. Brynger H, Persson H, Flatmark A, Albrechtsen D, Frödin L, Tufvesson G, Gäbel H, Weibull H, Möller E, Lundgren G, Groth CG (1988) No effect of blood transfusions or HLA matching on renal graft success rate in recipients treated with cyclosporine-prednisolone or cyclosporine-Azathioprine-prednisolone: the Scandinavian experience. Transplant Proc 20: 261–263
5. Carpenter CB, d'Apice AJF, Abbas AK (1976) The role of antibodies in the rejection and enhancement of organ allograft. Adv Immunol 2: 1–36
6. Cecka JM, Cicciarelli J, Mickey MR, Terasaki PI (1988) Blood transfusions and HLA matching. An either/or situation in cadaveric renal transplantation. Transplantation 45: 81–86
7. Chou S, Kim DY, Norman DJ (1987) Transmission of CMV by pretransplantation blood transfusion in renal transplantation candidate. J Infect Dis 155: 565–567
8. Es AA van, Marquet RL, Rood JJ van, Kalff MW, Balner H (1977) Blood transfusions induce prolonged kidney allograft survival in rhesus monkey. Lancet I: 506–508
9. Festenstein H, Sachs JA, Paris AMI, Pegrom GD (1976) Influence of HLA matching and blood transfusion on outcome of 502 London transplant group renal-graft recipients. Lancet I: 157–161
10. Hourmant M, Soulillou JP, Bui-Quang D, Guenel J (1979) The beneficial effect of blood transfusions: role of the time interval between the last transfusion and the transplantation. Transplantation 28: 40–44
11. Hourmant M, Babinet F, Cantarovich D, Latour M, Carcagne J, Vié H, Bonneville M, Moreau JF, Carosella E, Soulillou JP (1989) Polyclonal rabbit gammaglobulins against a human cytotoxic CD4+ T cell clone. II. Use in prevention of rejection in kidney transplantation: a pilot study. Transplantation 48: 260–263
12. Hourmant M, Taupin JL, Leymerigie F, Cantarovich D, Soulillou JP (1989) Azathioprine – cyclosporin A (CyA) double therapy versus CyA alone after the first rejection episode in kidney-transplanted patients under CyA. A randomized study. Transplant Int 2: 113–116
13. Iwaki Y, Cecka JM, Terasaki PI (1990) The transfusion effect in cadaveric kidney transplants – yes or no. Transplantation 49: 56–59
14. Kerman RH, Buren CT van, Lewis RM, Kahan BD (1988) Impact of blood transfusions and HLA on cyclosporine-treated renal transplant recipients. Transplant Proc 20: 264–269
15. Kerman RH, Buren CT van, Lewis RM, Kahan BD (1988) Successful transplantation of 100 untransfused cyclosporine-treated primary recipients of cadaveric renal allografts. Transplantation 45: 37–40
16. Marquet RL, Heysteck GA, Tinbergen WJ (1971) Specific inhibition of organ allograft rejection by donor blood. Transplant Proc 3: 708–711
17. Melzer JS, Husing RM, Feduska NJ, Tomlanovich SJ, Vincenti F, Amend WJC, Garovoy M, Salvatierra O Jr (1987) The beneficial effect of pretransplant blood transfusions in cyclosporine-treated cadaveric renal allograft recipients. Transplantation 43: 61–64
18. Muller JY, Kaplan C, Betuel H, Bignon JD, Fauchet R, Gluckman JC, Soulillou JP, Thibault P (1982) Effet des transfusions sanguines sur les greffes de rein. Nouv Presse Med 11: 3697–3701
19. Muller JY, Kaplan C, Cartron J (1982) Rapport de l'enquête Transfusion-Transplantation. Rev Fr Transfus Immunohematol 3: 327–352
20. Opelz G for the Collaborative Transplant Study (1987) Improved kidney graft survival in nontransfused recipients. Transplant Proc 19: 149–152
21. Opelz G, Terasaki PI (1976) Prolongation effect on blood transfusions of kidney graft survival. Transplantation 22: 380–382
22. Sanfilippo F, Vaughn WK, Bollinger RR, Spees EK (1984) The influence of pretransplant transfusions, using different blood products, on patient sensitization and renal allograft survival. Transplantation 37: 350–356
23. Soulillou JP, Bignon JD, Peyrat MA, Guimbretière J, Guenel J (1980) Systematic transfusions in hemodialysed patients awaiting grafts. Kinetics of anti-T and B lymphocyte immunization and its incidence on graft function. Transplantation 30: 285–289
24. Soulillou JP, Blandin F, Gunther E, Lemoine V (1984) Blood transfusion effect on heart allograft in rats. Studies of the role of the RT1 subregions. Transplantation 38: 63–67
25. Soulillou JP, Peyronnet P, Le Mauff B, Boeffard F, Godard A, Soulillou JP (1987) Prevention of rejection of kidney transplants by monoclonal antibody directed against interleukin 2 receptor. Lancet I: 1339–1342
26. Soulillou JP, Cantarovich D, Le Mauff B, Giral M, Hourmant M, Hirn M, Jacques Y (1990) Randomized trial of an anti-interleukin 2 receptor monoclonal antibody (33B3.1) versus rabbit antithymocyte globulin (ATG) in prophylaxis of early rejection in human renal transplantation. N Engl J Med 322: 1175–1182