20. Kuroda Y, Kawamura T, Suzuki Y, Fujiwara H, Yamamoto K, Saitoh Y. A new simple method for cold storage of the pancreas using perfluorochemical. Transplantation 1988; 46: 457.
21. Pegg D. Organ preservation. Surg Clin North Am 1986; 66: 617.
22. Idezuki Y, Feemster JA, Dietzman RH, et al. Experimental pancreaticoduodenal preservation and transplantation. Surg Gynecol Obstet 1968; 126: 1002.

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LONG-TERM BENEFIT OF INTRAVENOUS IMMUNOGLOBULINS IN CADAVERIC KIDNEY RETRANSPLANTATION

MARIE-NOËLLE PERALDI,[1] KODZO AKPOSSO, JEAN-PHILIPPE HAYMANN, ANTOINE FLAHAUT, CHRISTIANE MARLIN, ERIC RONDEAU, AND JEAN-DANIEL SRAER

Service de Néphrologie A and Association Claude Bernard, Hôpital Tenon, 75020 Paris, France

Renal retransplantation can be hampered by the presence of anti-HLA alloantibodies. Previous studies have documented in vitro and in vivo suppression of these antibodies by intravenous immunoglobulins (IVIg). We conducted a randomized study in 41 patients, who have received a second cadaveric transplant between 1989 and 1994. They all were treated with a quadruple-immunosuppressive protocol. In addition, 21 patients received 0.4 g/kg/day of IVIg, on the first 5 days after transplantation. The two groups of patients were identical for age, sex, duration of the first graft, duration of cold ischemia, anti-HLA sensitization, HLA matching, the number of acute rejection episodes, and the incidence of cytomegalovirus infection. The 5-year survival rate was significantly higher in the group of patients treated with IVIg: 68% versus 50% in the control group. The only significant factor associated with IVIg infusion and better survival was a shorter delay of graft function (3.4±1.0 days versus 9.9±1.6 days).

In conclusion, this randomized study demonstrates that IVIg treatment is associated with better long-term graft survival in retransplanted patients. This beneficial effect may be related to a long-lasting immunosuppressive effect of IVIg and/or to an early protective effect of the graft against ischemia.

An increasing number of patients returning to dialysis after renal transplantation are waiting for a second, a third, or even a fourth kidney transplant. The outcome of kidney cadaveric retransplantation is still a matter of debate. Graft survival for retransplanted patients (55–65% at 1 year) is substantially reduced compared with that of primary allograft recipients (usually higher than 75% at 1 year), even in the cyclosporine era (*1*, *2*). Many studies have tried to characterize the factors that influence the outcome of these retransplantations (*1–5*). Kidney retransplantation is often complicated by a high incidence of graft rejection, and this incidence is at least partly explained by anti-HLA immunization (*6*, *7*). Furthermore, transplantation can be delayed in immunized patients for months or years. The removal or suppression of these alloantibodies would, thus, help transplantation in these patients. In a previous study (*8*), we demonstrated that intravenous immunoglobulin (IVIg*) infusion combined with immunosuppressive drugs in sensitized primary renal transplant recipients could decrease the potency and the synthesis of anti-HLA antibodies, leading to a better graft survival. We, therefore, performed a randomized controlled study using prophylactic high-dose IVIg in second graft recipients. This study demonstrates that IVIg treatment is associated with a better graft survival and a significantly shorter delay of graft function.

From January 1989 to December 1994, 41 patients were included in the study. They all received a second kidney transplant of cadaveric origin. Initial kidney disease was of glomerular origin (21/41), vascular disease (9/41), interstitial nephropathy (6/41), urologic malformation (4/41), and polycystic kidney disease (1/41). All donor and recipient operations were performed according to standardized techniques, with implantation of the graft to the iliac fossa. The failed graft was removed only if infection or necrosis had occurred. Donor and recipient HLA typing, as well as the detection of panel-reactive IgG antibodies (PRA), were performed by France-Transplant. All transplantations were performed after negative T-cell and B-cell crossmatches. Crossmatches with historical sera were performed only for patients with PRA level >70%. Postoperative immunosuppressive regimens associated prednisolone, azathioprine, cyclosporine, and either antithymocyte globulins (ATG Fresenius, Munich, Germany) or OKT3 antibodies (Cilag, Beerse, Belgium). Rejection episodes were diagnosed by kidney biopsy and were treated by steroid pulses, antithymocyte globulins, and/or OKT3 antibodies. After randomization, 21 patients received IVIg infusion (0.4 g/kg/day) from day 0 to day 4 of transplantation. In both the group of patients treated with IVIg and in the control group, five patients received OKT3 instead of antithymocyte globulins. Over time, three different preparations of IVIg were used in this study: polyvalent IVIg prepared in the Centre National de Transfusion Sanguine of Paris (France), polyvalent IVIg prepared in the Centre National de Transfusion Sanguine of Lille (France), and commercially manufactured Gamma-Gard (Baxter). All the preparations were treated to inactivate viruses. The presence of soluble HLA molecules or anti-HLA antibodies was not specifically determined in this study, but IVIg preparations from different sources have been shown to contain little amounts of soluble HLA molecules or anti-HLA antibodies (*9*).

The studied parameters included the following: (1) age; (2) sex; (3) duration of the first graft; (4) duration of cold isch-

[1] Address correspondence to: M.N. Peraldi, Service de Néphrologie A, Hôpital Tenon, 4 rue de la Chine, 75020 Paris, France.

* Abbreviations: CMV, cytomegalovirus; IVIg, intravenous immunoglobulins; PRA, panel-reactive IgG antibodies.

emia; (5) the delay of graft function (i.e., the number of days between surgery and urinary output greater than one liter/day); (6) anti-HLA sensitization; (7) HLA matching; (8) the number of acute rejection episodes diagnosed by kidney biopsy; and (9) the incidence of cytomegalovirus (CMV) infection, as detected by viremia, fever, and/or hepatic and pulmonary infection.

Survival was calculated using the Kaplan-Meier actuarial analysis, with *P*-values generated by the log rank analysis. All patients with a functioning graft had a minimum of 20-month follow-up (range 20–65 months). Graft loss was considered to have occurred at the time of return to dialysis or of death with a functioning graft. There was no exclusion for technical or nonimmunologic failures.

Variables between groups were compared with a *t* test. Results are expressed as mean±SEM.

Clinical characteristics of the patients were as follows. Twenty-one patients, who received prophylactic IVIg, were compared to the 20 patients who received only the conventional quadruple-immunosuppressive therapy. In each group, the same number of patients received OKT3 antibodies instead of antithymocyte globulins. As shown in Table 1, there were no differences between the two groups in age, sex, HLA matching, peak PRA level before second transplantation, PRA level at the time of transplantation and 1 month after retransplantation, cold ischemia, or the number of acute rejection episodes. In the two groups of patients, HLA immunization was important because a PRA level greater than 30% was present in 53% of patients before their second transplantation, whereas this incidence was only 15% for the same patients before their first transplantation. No patient had HLA-A or -B repeated mismatches. Only one patient in each group had a repeated DR mismatch (DR7 in one patient receiving IVIg and DR6 in one patient of the control group). The duration of the first transplant (with a cut-off value of 1 year) did not significantly influence the duration of the second transplant ($P=0.31$ Cox model), although there was a trend for shorter second graft duration when the first graft was lost during the first 3 months (not shown). The causes of first graft loss were identical in the two groups of patients, as shown in Table 1. The only significantly different factor between the two groups was a shorter delay of graft function: 3.4 days in the IVIg group compared with 9.9 days in the group receiving no IVIg. Extracellular volume expansion during the first 24 hr after transplantation was assessed by the percentage of increase in body weight. In the two groups, increase in body weight was not significantly different: 8.53±1.15% in the group treated with IVIg and 8.40±1.11% in the control group. In both groups, CMV infection occurred with the same incidence: 54% of the patients receiving IVIg and 60% of the patients in the control group.

Graft and patient survival rates were compared between the IVIg-treated and control groups. Kaplan-Meier analysis documented a better actuarial graft survival rate, which was significantly higher ($P=0.0017$ log rank) in the patients treated with IVIg (Fig. 1). The 5-year survival rate of the grafts was 68% in the group of patients receiving IVIg, and only 50% in the control group. This graft survival rate in retransplanted patients treated with IVIg is not statistically different from the survival rate of single kidney transplant recipients grafted during the same period in our unit (72% at 5 years; not shown).

Patient survival was not different in the two groups, with a 5-year survival rate of 90% in the group of patients receiving IVIg and 95% in the control group (Fig. 2).

This work demonstrates for the first time the beneficial effects of IVIg, through a randomized study in retransplanted patients receiving kidneys of cadaveric origin. The benefit of IVIg appears early in the post-transplantation course and is prolonged over several years. In the two groups

TABLE 1. Clinical characteristics of the patients treated (+IVIg) or not (−IVIg) by intravenous immunoglobulins the first 5 days after retransplantation

	+IVIg (n=21)	−IVIg (n=20)	
Age	36.4±1.9	34.1±2.2	NS
Sex (M/F)	17/4	15/5	
HLA-A, -B, -DR identities	2.2±1.2	2.1±1.0	NS
No. of patients with repeated mismatches	1/21	1/21	
Peak PRA level before second graft	39±9	38±10	NS
PRA level (%) at the time of second graft	32±7	36±8	NS
No. of patients with PRA level >80% at the time of second graft	4/21 (19%)	4/20 (20%)	
PRA level (%) 1 mo after second graft	6±2	7±3	NS
No. of acute rejection episodes	2.1±1.1	2.2±1.1	NS
Cold ischemia (hr)	28.9±1.5	29.5±1.6	NS
Delay of graft function (days)	3.4±1.0	9.9±1.6	$P<0.002$
Percentage of patients with CMV infection (no. of patients)	57 (12/21)	60 (12/20)	NS
Causes of first graft loss			NS
Surgical complications/sepsis	n=1	n=2	
Irreversible acute rejection	n=3	n=3	
Repeated acute rejection episodes/chronic rejection	n=17	n=15	

Values are expressed as mean±SEM, except where noted. NS, nonsignificant.

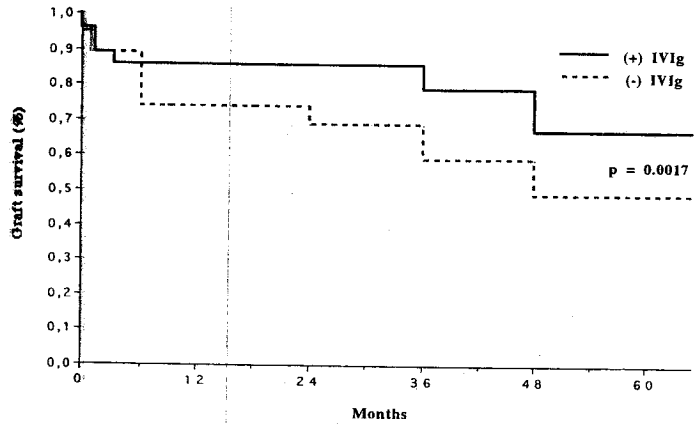

FIGURE 1. Kidney actuarial survival rate of retransplanted patients treated (——) or not treated (- - - -) with IVIg (Kaplan-Meier analysis).

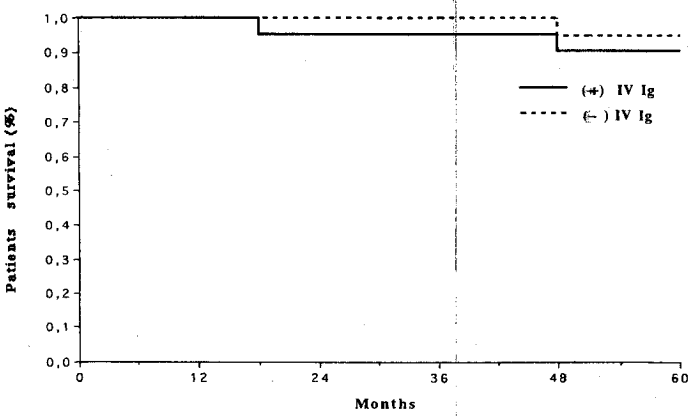

FIGURE 2. Survival of retransplanted patients treated (——) or not treated (- - - - -) with IVIg (Kaplan-Meier analysis).

of patients, there was no difference in HLA immunization as measured by PRA level, either before or at the time of second graft. Our patients were high-risk candidates for retransplantation, because a PRA level greater than 30% was present in 6 of the 41 patients (15%) before their first transplantation, and in 22 of 41 (53%) before their second transplantation. The number of acute rejection episodes, as well as the duration of cold ischemia, was not different in IVIg-treated or control patients. We did not find any significant influence of the duration of the first graft on the second graft survival, but the number of patients was too small to statistically determine the influence of the first graft survival, especially in the group of patients with a first graft duration shorter than 3 months. The present study confirms our previous nonrandomized study, which demonstrated that IVIg given to sensitized primary renal transplant recipients was associated with a very good graft survival (95% at 1 year) in a high-risk group of patients (*8*).

However, the mechanism of action of IVIg is not yet well established. Presumably, the most important mechanism is the immunomodulatory role of IVIg. IVIg have been extensively used in autoimmune (*10, 11*) and alloimmune disorders (*12*). Alloantibodies are produced after blood transfusions, graft failure, and pregnancy, with a synergistic effect of any combination of these factors (*13*). It has been demonstrated in vitro and in vivo that IVIg suppress HLA alloantibodies in highly sensitized transplant candidates, with a decrease in PRA level and a good tolerance for a histoincompatible organ (*14, 15*). The presence of anti-idiotypic antibodies to HLA in IVIg (*16, 17*) may be relevant in vivo in their immunoregulatory effects. In our study, the decrease in PRA level is important after IVIg treatment, but is not significantly different from the decrease in PRA level observed in the control group. This decrease in PRA level is due to the immunosuppressive therapy given for the transplantation, and thus, IVIg efficiency cannot be assessed on this parameter. The nonspecific antiinflammatory role of IVIg may be of importance, because it has been demonstrated that IVIg can modulate HLA antigen expression (*9*), neutralize circulating HLA antibodies (*15*), block phagocytic cells (*18*), and inhibit cytokines and complement (*19, 20*).

We found a shorter delay of graft function after IVIg, as compared with patients without IVIg, whereas the duration of cold ischemia was not different in the two groups. A recent study has documented a beneficial effect of intravascular volume expansion using intraoperative albumin administration: transplant recipients given high-dose (1.2–1.6 g/kg) albumin have a significantly lower incidence of delayed graft function (*21*). Thus, IVIg infusion may shorten the delay of graft function through an improvement in graft perfusion, as it is now well documented that medullary ischemia of the kidney is associated with delayed graft function, which itself is correlated to chronic rejection (*22–24*). However, this potential "hemodynamic" effect does not rule out an immunological mechanism. One can speculate that anti-HLA antibodies at low concentration at the time of transplantation may deposit in the kidney, leading to renal injury and delayed graft function. IVIg may, therefore, be beneficial by preventing the binding of anti-HLA antibodies.

In conclusion, this randomized study demonstrates the clinical efficacy of IVIg in kidney retransplantation, with a significant shorter delay in graft function and a long-term improvement in graft survival. Further studies are needed to determine the respective importance of initial renal perfusion, immunomodulation, and antiinflammatory properties of IVIg in the mechanisms of graft protection.

REFERENCES

1. Gaston RS, Shroyer TW, Hudson SL, et al. Renal retransplantation: the role of race, quadruple immunosuppression, and the flow cytometry crossmatch. Transplantation 1994; 57: 47.
2. Stratta RJ, Oh CS, Sollinger HW, Pirsch JD, Kalayoglu M, Belzer FO. Kidney retransplantation in the cyclosporine ERA. Transplantation 1988; 45: 40.
3. Reed E, Hardy M, Benvenisty A, et al. Effect of anti-idiotypic antibodies to HLA on graft survival in renal allograft recipients. N Engl J Med 1987; 316: 1450.
4. Ascher NL, Ahrenholz DH, Simmons RL, Najarian JS. One hundred second renal allografts from a single transplantation institution. Transplantation 1979; 27: 30.
5. Deierhoi MH, Sollinger HW, Kalayoglu M, Belzer FO. Quadruple immunosuppression in 305 consecutive cadaver renal allografts. Clin Transplant 1987; 1: 71.
6. Spees K, Vaughn WK, McDonald JC. Why do secondary cadaver renal transplants succeed? J Urol 1983; 129: 484.
7. Sanfilippo F, Vaughn WK, Bollinger RR, Spees EK. Comparative effects of pregnancy, transfusion, and prior graft rejection on sensitization and renal transplant results. Transplantation 1982; 34: 360.
8. Rondeau E, Fournier A, Kanfer A, Sraer JD. Polyvalent immunoglobulins in sensitised renal transplant recipients. Nephrol Dial Transplant 1991; 6: 675.
9. Kaveri S, Vassilev T, Hurez V, et al. Antibodies to a conserved region of HLA class I molecules, capable of modulating CD8 T cell-mediated function, are present in pooled normal immunoglobulin for therapeutic use. J Clin Invest 1996; 97: 865.
10. Fehr J, Hoffmann V, Kappeler U. Transient reversal of thrombocytopenia in idiopathic thrombocytopenic purpura by high-dose intravenous gammaglobulin. N Engl J Med 1982; 306: 1254.
11. Bussel J, Lacezari P, Hilgartner M, et al. Reversal of neutropenia with intravenous gammaglobulin in auto-immune neutropenia of infancy. Blood 1983; 62: 398.
12. Sultan Y, Kazatchkine MD, Maisonneuve P, Nydegger VE. Antiidiotypic suppression of autoantibodies to factor VIII (antihaemophilic factor) for high-dose intravenous gammaglobulin. Lancet 1984; 120: 765.
13. Reed E, McNamus P, Jo E, et al. Prediction of humoral immune responses to HLA through antiidiotypic antibody studies.

Transplant Proc 1989; 21: 708.
14. Tyan DB, Li VA, Czer L, Trento A, Jordan SC. Intravenous immunoglobulin suppression of HLA alloantibody in highly sensitized transplant candidates and transplantation with a histoincompatible organ. Transplantation 1994; 57: 553.
15. Glotz D, Haymann JP, Sansonnetti N, et al. Suppression of HLA-specific alloantibodies by high-dose intravenous immunoglobulins (IVIg). Transplantation 1993; 56: 335.
16. Rossi F, Dietrich G, Kazatchkine MD. Anti-idiotypes against autoantibodies in normal immunoglobulins: evidence for network regulation of human autoimmune responses. Immunol Rev 1989; 110: 135.
17. Haymann J Ph, Glotz D, Sansonnetti N, François A, Druet Ph, Bariéty J. Normal human polyclonal immunoglobulins for intravenous use (IVIg) contain anti-idiotypes directed against human anti-HLA antibodies. J Am Soc Nephrol 1993; 4: 939.
18. Ruiz de Souza V, Carreno MP, Kaveri SV, et al. Selective induction of interleukin-1 receptor antagonist and interleukin-8 in human monocytes by normal polyspecific IgG (intravenous immunoglobulin). Eur J Immunol 1995; 25: 1267.
19. Andersson UG, Bjork L, Skansen-Saphir U, Andersonn JP. Down regulation of cytokine production and IL2 receptor expression by pooled human IgG. Immunology 1993; 79: 211.
20. Basta M, Dalakas MC. High-dose intravenous immunoglobulin exerts its beneficial effect in patients with dermatomyositis by blocking endomysial deposition of activated complement fragments. J Clin Invest 1994; 94: 1729.
21. Dawidson I, Sandor ZF, Coorpender L. Intraoperative albumin administration affects the outcome of cadaver renal transplantation. Transplantation 1992; 53: 774.
22. Peters TG, Shaver TR, Ames JE IV, Santiago-Delpin EA, Jones KW, Blanton JW. Cold ischemia and outcome in 17,937 cadaveric kidney transplants. Transplantation 1995; 59: 191.
23. Suthanthiran M, Strom TB. Renal transplantation. N Engl J Med 1994; 331: 365.
24. Brezis M, Rosen S. Hypoxia of the renal medulla: its implications for disease. N Engl J Med 1995; 332: 647.

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DO COLD AGGLUTININS HAVE ANY IMPACT ON THE OUTCOME OF LIVER TRANSPLANTATION?

WILLEM A. MARSMAN,[1] S. BREANNDAN MOORE,[2] MARIO KONDO,[1] KEVIN L. BUNDY,[2] GREGORY J. GORES,[1] RUSSELL H. WIESNER,[1] AND RUUD A. F. KROM[1,3]

Divisions of Liver Transplantation and Transfusion Medicine, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905

Cold agglutinins, IgM red blood cell autoantibodies, cause cold agglutinin disease with hemolysis and microvascular occlusion. Cold preservation of kidneys during renal transplantation in the presence of cold agglutinins can cause graft malfunction. However, the impact of cold agglutinins on the outcome of liver transplantation is unknown. We measured the pretransplant presence and titer of cold agglutinins in 327 primary liver allograft recipients and analyzed their relationship to outcome after transplant. Thirty-three percent of pretransplant patients had cold agglutinins. Cold agglutinins were more common in patients with viral-related liver diseases (49%) compared with those with nonviral-related liver disease (32%). There was no difference between recipients with and without cold agglutinins in usage of blood products, postoperative day 2 aminotransferase levels, acute rejection at day 7, the development of hepatic artery thrombosis, nonanastomotic biliary strictures, or 4-month allograft survival. In conclusion, cold agglutinins are common in liver transplant patients before surgery, especially those with viral-related liver diseases. However, the presence of cold agglutinins does not impact on outcome after liver transplantation.

Cold agglutinins are IgM red blood cell autoantibodies that react most strongly at 0–5°C. They are commonly found in normal individuals with a titer of less than 1/64 at 4°C. Occasionally they are found in high titers (>1/1000 in saline), reacting in a much broader temperature range with reactivity up to 30°C or even 37°C. In this situation, hemolysis can occur as part of the cold agglutinin disease, which can be accompanied by acrocyanosis and microvascular occlusions (*1*).

In cardiac surgery using hypothermia, cold agglutinins can lead to complications, and screening of patients for these antibodies has been recommended (*2*). In solid organ transplantation using cold preservation, the presence of cold agglutinins in the recipient could theoretically lead to microvascular injury after implantation and reperfusion. Indeed, failure of renal allografts due to the presence of cold agglutinins has been reported (*3, 4*). The impact of cold agglutinins on the morbidity and mortality after liver transplantation is unknown, as the experience is limited to two case reports (*5, 6*).

We hypothesized that in liver transplantation, the presence of cold agglutinins in the recipient might contribute to ischemic damage of the graft, leading to dysfunction, and in particular, to the development of biliary strictures. Therefore, we measured the pretransplant presence and titer of cold agglutinins in liver allograft recipients and analyzed their relation to outcome of the graft and incidence of type biliary strictures.

A total of 329 patients, who received first liver allografts between 1989 and 1994, were included in this study. Pretransplant serum samples of these patients were retrospectively analyzed at 4°C for the presence of cold agglutinins,

[1] Division of Liver Transplantation.
[2] Division of Transfusion Medicine.
[3] Address correspondence to: Ruud A. F. Krom, MD, PhD, Division of Liver Transplantation, Mayo Clinic, 200 First Street SW, Rochester, MN 55905.