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REVERSAL OF STEROID- AND ANTI-LYMPHOCYTE ANTIBODY-RESISTANT REJECTION USING INTRAVENOUS IMMUNOGLOBULIN (IVIG) IN RENAL TRANSPLANT RECIPIENTS

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Background. **Despite the recent advances in immunosuppression, steroid-resistant rejection remains a difficult problem in renal transplant recipients.**

Methods. **We reviewed our experience with i.v. immunoglobulin (IVIG) in the treatment of steroid- and antilymphocyte antibody-resistant rejection in renal transplant patients. Between September 1996 and March 1999, 17 patients were treated with IVIG to reverse steroid- or antilymphocyte antibody-resistant rejection. A total of 2 g/kg of IVIG was administered to patients during each treatment course.**

Results. **With a mean follow-up of 21.5±9.5 months from the time of IVIG administration, patient and graft survival rates were 94% (16/17) and 71% (12/17), respectively. The baseline mean serum creatinine level prior to rejection was 2.2±0.7 mg/dl and peaked at 3.3±1.1 mg/dl at the time of the diagnosis of refractory rejection. IVIG therapy was associated with a fall in the mean creatinine to 2.8±1.1 mg/dl. The most recent serum creatinine in patients with functioning grafts was 2.8±1.6 mg/dl. In 82% of allograft biopsies after IVIG, reversal or reduction in the severity of rejection was demonstrated. In addition, IVIG therapy rescued three of four patients with antilymphocyte antibody-resistant rejection.**

Conclusions. **IVIG rescue therapy for steroid- or antilymphocyte antibody-resistant rejection is associated with resolution or improvement of rejection severity, stable renal function, and reasonable graft survival.**

INTRODUCTION

Although immunosuppression after renal transplantation has become increasingly powerful in recent years, rejection remains an important complication. Steroid-resistant rejection is an especially significant problem, and despite the availability and efficacy of antilymphocyte antibody therapy, remains associated with accelerated rates of renal allograft loss (*1*). Apart from mycophenolate mofetil and tacrolimus rescue therapy, few other treatment modalities have been able to reverse steroid-resistant rejection (*2*, *3*).

In the past, i.v. immunoglobulin (IVIG) has been used to treat autoimmune disorders (*4*), as well as infectious diseases in immunosuppressed patients (*5*). Recently, it has been used in transplantation to prevent graft versus host disease (GVHD) in bone marrow transplant recipients (*6*), and to reduce anti-HLA antibodies in sensitized patients awaiting organ transplantation (*7*). There is also preliminary evidence that it can be used as an induction agent (*8*, *9*), and that it may be able to reverse antibody-mediated rejection in the early posttransplant period (*10*). However, the data regarding the clinical use of IVIG to reverse steroid-resistant rejection are extremely limited (*10–12*), and to date, no report has addressed its use in treatment of antilymphocyte antibody-resistant rejection. In this study, we report the University of Pittsburgh experience with IVIG in reversing steroid- and antilymphocyte antibody-resistant rejection in renal transplant recipients.

PATIENTS AND METHODS

Between September 1996, and March 1999, 25 patients received IVIG for steroid- or antilymphocyte antibody-resistant rejection at the University of Pittsburgh (the preparation used was Sandglobulin, which had been selected by the hospital independently of any input from the transplant service). Eight patients were excluded from analysis because of concurrent administration of antilymphocyte antibody therapy; thus, a total of 17 patients were analyzed (9 male, 8 female). All patients had biopsy-proven rejection, and all had post-IVIG allograft biopsies to document treatment efficacy. Thirteen patients (76%) were treated for steroid-resistant rejection, and 4 patients (24%) were treated for antilymphocyte antibody-resistant rejection. Patient demographics are shown in Table 1.

Significantly, these patients were not believed to be at an increased risk for antibody-mediated hyperacute or accelerated rejection. Rejection was not suspected during the first postoperative week in any of the 17 patients, and the time from transplantation to

TABLE 1. Patient demographics

n	17 (100%)
Mean age (yr)	43.9±15.0 yr (range 26–72)
Gender	9 male: 8 female
% PRA (DTT)	12.5±22.3 (range 0–72)
Number of prior rejection episodes with current graft	2.0±1.6 (range 0–6)
Second renal transplant	5 (29%)
Third renal transplant	1 (6%)
Living-related renal transplant	2 (12%)
Previous liver transplant	2 (12%)
Patients failing antilymphocyte therapy	4 (24%)

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commencement of IVIG therapy was 17.5±23.7 months (range 1–84). The mean % PRA was 12.5±22.3 (range 0–22); six patients (35%) had previously received a renal allograft.

Sixteen (94%) patients had received tacrolimus-based immunosuppression. The remaining patient received microemulsion cyclosporine. Six (35%) patients received mycophenolate mofetil maintenance therapy, and six (35%) patients had been weaned off corticosteroids prior to the development of refractory rejection.

A total of 2 g/kg of IVIG was administered over 2 to10 days for each treatment course, according to the fluid balance status of each patient. Four (24%) patients received two courses of IVIG, and three (18%) patients received three or more courses. The mean tacrolimus dose was increased by 1.5±2.4 mg/d as part of the treatment for refractory rejection. The IVIG course was accompanied by a steroid recycle in 10 patients, and in 7 patients, mycophenolate mofetil was added (mean dose 1143±690 mg/day, range 500–2000 mg/day).

RESULTS

The mean follow-up was 39.0±23.1 months from the time of transplantation and 21.5±9.5 months from the time of IVIG therapy. The patient survival rate was 94% (16/17), and the graft survival rate was 71% (12/17). The sole mortality involved a patient who had had a liver transplant 7 years previously. She developed fungal endocarditis 13 months after IVIG therapy and died. Four graft losses (80%) were attributed to chronic rejection, and the remaining graft loss was related to a failure of IVIG rescue therapy.

Renal allograft function was reasonably well maintained after IVIG therapy. The baseline serum creatinine level before development of rejection was 2.2±0.7 mg/dl, and rose to 3.3±1.1 mg/dl at the time of the refractory rejection episode. IVIG therapy was associated with a reduction in the mean serum creatinine 2 weeks after the conclusion of therapy (2.8±1.1 mg/dl). The current serum creatinine in patients with functioning grafts is 2.8±1.6 mg/dl.

Before the initiation of IVIG therapy, 47% (8/17) of patients had Banff IA, 29% (5/17) had Banff IB, and 24% (4/17) had Banff II rejection. After IVIG therapy, 53% of allograft biopsies (9/17) demonstrated complete resolution of rejection, and 29% (5/17) demonstrated reduced rejection severity. Overall, 82% of allograft biopsies had a reduction in rejection severity. Surprisingly, the severity of rejection prior to IVIG therapy did not predict treatment outcome (Fig. 1).

As a number of our patients were treated concomitantly with a steroid recycle or mycophenolate mofetil, it was difficult to analyze the impact of IVIG by itself on refractory rejection. Thus, we analyzed a subset of seven patients who received IVIG without any other adjunctive therapy. The demographics of patients receiving IVIG alone were similar to those in patients receiving mycophenolate mofetil and/or a steroid recycle in addition to IVIG (Table 2). Of these seven patients who received IVIG monotherapy, three (43%) had Banff IA, two (29%) had Banff IB, and two (29%) had Banff II rejection before the initiation of therapy. Six of seven (86%) post-IVIG allograft biopsies in these patients demonstrated a reduction in or resolution of rejection. The mean baseline serum creatinine level in these patients was 2.2±0.9 mg/dl, rose to 3.7±1.2 mg/dl at the time of rejection, and fell to 2.7±1.3 mg/dl 2 weeks after IVIG therapy. The current mean serum creatinine in the six patients with functioning allografts is 3.0±2.1 mg/dl. Thus, IVIG by itself appeared to be able to reverse refractory rejection.

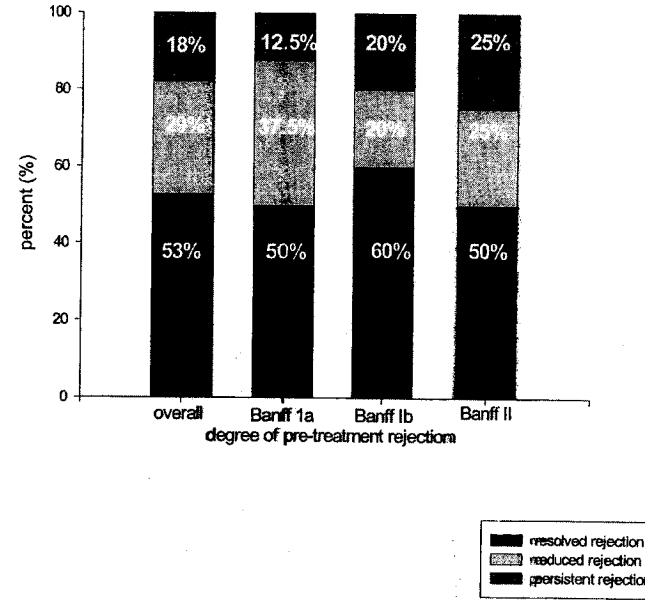

FIGURE 1. Reversal of rejection after administration of IVIG.

Four patients received IVIG to reverse antilymphocyte antibody-resistant rejection. Three patients had failed OKT3 therapy, and one patient had failed anti-thymocyte globulin (ATG) therapy for either steroid-resistant or Banff II rejection. Allograft biopsy at the time of anti-lymphocyte antibody-resistant rejection demonstrated Banff Ia in two patients, Banff Ib in one patient, and Banff II rejection in the remaining patient. IVIG was able to completely reverse rejection in one patient and reduce rejection severity to borderline rejection in two other patients. Overall, IVIG rescued three of four patients with antilymphocyte antibody-resistant rejection. IVIG treatment lowered the mean serum creatinine from 3.0±1.3 mg/dl at the time of refractory rejection to 2.5±1.1 mg/dl 2 weeks after the completion of treatment. The current mean serum creatinine is 2.0±1.2 mg/dl in the three patients with functioning allografts.

Complications after IVIG therapy were rare. Having failed ATG, one patient received IVIG and developed symptomatic cytomegalovirus (CMV) infection 5 months after treatment of refractory rejection. Another patient, status post liver transplantation 7 years earlier, developed fungal endocarditis 13 months after IVIG therapy. It is likely that these infectious complications were related to intensive long-term immunosuppression. IVIG was well tolerated, and no complications could be directly attributed to IVIG therapy.

DISCUSSION

Pooled human gammaglobulin, IVIG, has been used to treat infectious complications in immunosuppressed patients (*5*), autoimmune idiopathic thrombocytopenic purpura (*4*), and vasculitis (*13*) since the early 1980s. Only recently has it been used in bone marrow and solid organ transplantation. IVIG therapy is associated with a lower incidence of GVHD in bone marrow transplant recipients (*6*). Its ability to reduce the levels of anti-HLA antibodies has enabled sensitized patients with prohibitively high PRAs to be transplanted (*7*). IVIG has also been used in induction therapy (*8*, *9*) and has

TABLE 2. Demographics of patients receiving IVIG monotherapy versus IVIG with adjunctive therapy

Category	IVIG alone	IVIG with adjunctive therapy	Significance
No.	7	10	
Adjunctive therapy	None	MMF and/or steroid recycle	
Gender	4 male/3 female	5 males/5 females	NS
Age (yr)	41.3±16.8 (26–72)	45.7±14.1 (30–71)	NS
% PRA	18.1±30.3 (0–82)	7.3±13.3 (0–36)	NS
Time from transplant to IVIG therapy (mo)	17.3±30.3 (1–84)	17.6±19.7 (1–58)	NS
Previous kidney transplant	3 (43%)	3 (30%)	NS
Previous liver transplant	2 (29%)	0 (0%)	NS
Living-related transplant	1 (14%)	1 (10%)	NS
Patients failing antilymphocyte therapy	1 (14%)	3 (30%)	NS

NS, Not significant ($P>0.05$).

demonstrated the ability to reverse antibody-mediated rejection (*10—12*).

Although IVIG has been shown to be able to block anti-HLA antibodies via antiidiotype antibodies (*14*), the mechanism by which it reverses established non-HLA antibody-related rejection is unclear. Several theories have been proposed. Marchalonis et al. proposed that antiidiotype antibodies from IVIG can bind to the hypervariable region of the T cell receptor, and thereby inhibit T cell-mediated rejection (*15*). IVIG has also been thought to provide anti-CD4 activity (*16*) and block cytokine receptors (*17*). Other in vitro studies have shown that IVIG has been shown to down-regulate both T and B cell activation and antibody production (*18*), as well as suppress tumor necrosis factor (TNF) production (*17*). Recent in vivo studies have demonstrated the ability of IVIG to prolong xenograft survival (*19*).

Casadei et al. presented data suggesting that IVIG can rescue up to 82% of grafts with steroid-resistant rejection (*11, 12*). Our data support their findings. With a follow-up of 21.5 months, both graft and patient survival were relatively good, with maintenance of stable renal allograft function. For the first time, we also demonstrated that IVIG was able to reverse anti-lymphocyte antibody-resistant rejection.

A number of patients in our study were treated concomitantly with a steroid recycle and initiation of mycophenolate mofetil, confounding our ability to assess the efficacy of IVIG in reversing rejection. However, in a subgroup of patients who did not receive additional concurrent antirejection therapy, rejection was completely eliminated or markedly reduced in 6 of 7 patients. Therefore, IVIG therapy by itself appears to be able to reverse steroid-resistant rejection.

We included the patients that received steroid recycles and/or mycophenolate mofetil in conjunction with IVIG therapy in our analysis to determine whether the addition of adjunctive therapy was associated with improved or reduced treatment efficacy, and whether this combination was associated with an unacceptably high complication rate. Eight of 10 patients (80%) who were treated with adjunctive therapy demonstrated a reduction in rejection severity, compared to 6 of 7 patients (86%) in the IVIG monotherapy group. The complication rates in both groups were similar. One of 7 patients (14%) in the IVIG monotherapy group developed fungal endocarditis 13 months post-IVIG therapy, and 1 of 10 patients (10%) in the adjunctive group developed CMV 5 months post-IVIG. Again, neither of these complications could be attributed directly to IVIG, and were likely secondary to intensive immunosuppressive therapy both before and after IVIG therapy.

It appears that the efficacy of IVIG therapy in the treatment of steroid-resistant rejection may be similar to that of OKT3 or ATG. The main advantage of IVIG over antilymphocyte therapy is the relative paucity of side effects. In addition, its inherent antiviral properties make IVIG an attractive agent in treating rejection in patients who are at high risk for immunosuppression-related viral infections, such as CMV. However, despite these antiviral properties, one patient developed CMV enteritis 5 months post-IVIG therapy. We attributed this complication to intensive immunosuppressive therapy that included a course of ATG before the use of IVIG. It appears that IVIG does not confer complete prophylaxis against CMV when given shortly after the conclusion of ATG therapy.

The cost of IVIG therapy is an important issue. Although one course of IVIG is currently more expensive than antilymphocyte antibody therapy, IVIG can be given in an outpatient setting, without the need for continuous monitoring or central venous access. Ultimately, prospective, randomized studies will be required to evaluate the efficacy, optimal therapeutic dose, and relative cost of IVIG in the treatment of organ transplant rejection.

In conclusion, our data suggest that IVIG rescue therapy for steroid-resistant rejection is associated with histological resolution or improvement of rejection severity, maintenance of renal function, and long-term graft survival. In addition, it seems that IVIG is capable of reversing antilymphocyte antibody-resistant rejection.

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MEASUREMENT OF MYCOPHENOLATE MOFETIL EFFECT IN TRANSPLANT RECIPIENTS[1]

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Background. **Immunosuppression involves the nature of the immunosuppressive agents and individual differences in patient factors. We investigated whether the effect of mycophenolate mofetil (MMF) is measurable using an in vitro measure of immunocompetence and related its effects to cyclosporine (CsA) in vitro.**

Methods. **Liver or kidney transplant recipients receiving prednisone; CsA or tacrolimus; and MMF, azathioprine (AZA), or neither, were studied. Immunocompetence was assessed by one-way mixed lymphocyte culture using patients' peripheral blood leukocytes (PBL) and three validated stimulators. The effect of immunosuppressive agents added in vitro on normal PBL stimulation by *Staphylococcus* enterotoxin B was determined by the carboxyfluorescein diacetate succinimidyl ester measurement of division.**

Results. **Patients receiving MMF had an average immunocompetence level of 12±23, compared with 39.7±65 and 25.5±42 for those receiving AZA or neither AZA nor MMF, respectively. Thus, there was an approximately 80% suppression of the response in the MMF group. Assessment of normal cell division revealed that CsA allowed multiple cell generations but suppressed the numbers of cells in each, whereas MMF blocked proliferation into subsequent generations. Addition of clinically relevant levels of mycophenolic acid, the active agent for MMF, added to more moderate levels of CsA, was required to achieve greater than 80% suppression, consistent with the degree of immunocompetence depression measured in patients.**

Conclusions. **These data provide the novel finding that the effect of MMF treatment on patients is measurable in their PBL as decreased immunocompetence in vitro. The effect of MMF on normal PBL approximates the 80% inhibition that we found in patients. Moreover, the effect of MMF on cell division provides**

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