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Antithymocyte globulin for steroid resistant rejection in renal transplant recipients immunosuppressed with triple therapy

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Abstract. Steroid resistant rejection, confirmed histologically, occurred in 35 of 187 consecutive cadaveric renal transplants treated with triple therapy (cyclosporin, azathioprine and prednisolone) in the Oxford Transplant Unit. Twenty-seven of these were treated with a rabbit antithymocyte globulin (ATG) and 19 showed recovery of function. The level of serum creatinine, the renal biopsy appearance and the requirement for dialysis at the start of ATG treatment did not predict which patients would respond to the therapy. One year after transplantation there was no significant difference between the mean plasma creatinine levels of those patients with steroid resistant rejection who had been given ATG and responded (151.6 μmol/l) and those who had responded to steroids alone (165.0 μmol/l). Adverse effects of ATG treatment included a mean fall in white cell count of 62.2% and a mean fall in platelet count of 45.1%. Two of the 27 patients who received ATG died (7.4% mortality). ATG would appear to be an effective treatment of steroid resistant rejection in patients receiving triple therapy immunosuppression, and graft function may subsequently be excellent in those patients who respond to treatment.

Key words: Antithymocyte globulin - Triple therapy in kidney transplantation - Immunosuppression - Steroid-resistant rejection.

The use of cyclosporin in renal transplantation has been associated not only with improved allograft survival, but also with reduction in the frequency and severity of rejection episodes compared with treatment using prednisolone and azathioprine [2, 7, 16]. In an attempt to reduce the incidence of the side effects of cyclosporin while maintaining improved graft survival, many units have begun to use triple therapy with low-dose cyclosporin, azathioprine and prednisolone [11, 12, 16, 19, 20].

Despite these advances in immunosuppression, allograft rejection that is resistant to treatment with high-dose intravenous or oral steroids still occurs and may result in graft loss. Antithymocyte globulin (ATG) has been used successfully to treat steroid resistant rejection in patients treated with prednisolone and azathioprine, and also in patients treated with cyclosporin [3, 4, 14], but its efficacy and safety when used in conjunction with triple therapy have not previously been described. In this report we present our experience with a rabbit ATG used for the treatment of steroid resistant rejection in patients receiving triple therapy.

Patients and methods

Between May 1985 and May 1988, 187 consecutive cadaveric renal transplants were performed in 179 adult patients. Triple therapy with cyclosporin, azathioprine and prednisolone was used in all patients. The details of therapy and results for the first 100 patients have previously been reported [12]. The results of living related transplants have not been included in this study; two of these patients have been treated with ATG for steroid resistant rejection with subsequent recovery of graft function.

Triple therapy immunosuppression consisted of 4 mg/kg per day cyclosporin intravenously for 4 days after transplantation and then 10 mg/kg per day with the dose adjusted to obtain trough whole blood levels in the range of 400-800 ng/ml; intravenous cyclosporin was not used in the first 45 consecutive patients, who received oral cyclosporin as a single dose of 10 mg/kg preoperatively and 10 mg/kg per day thereafter. Azathioprine was given at a dose of 1.5 mg/kg per day; this was reduced if the total peripheral white cell count fell below 4.0×10^9/1. Prednisolone was given at a dose of 10 mg twice daily for the first 90 days; thereafter it was reduced to 5 mg twice daily. High-risk patients (defined as those with a current positive crossmatch or current negative but historical positive crossmatch, or those with greater than 90% panel reactivity) were given prophylactic intravenous methylprednisolone at

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Table 1. Details of 187 consecutive cadaveric transplants in this series

Total number of cadaveric grafts	187
First grafts	145
Second or subsequent grafts	42
Number with acute rejection episodes in first 3 months after transplant	140
Mean number of courses of steroids per patient with acute rejection in first 3 months after transplant	2.3
Number with steroid resistant rejection	35
Treated with ATG	27 (8 failed)
Not treated with ATG	8 (8 failed)

Table 2. Characteristics of patients who responded or who failed to respond to ATG

	Number of responders	Number of non-responders
Total	19	8
Male	11	7
Mean plasma creatinine at start of treatment (μmol/l)	574	540
DR matching		
0 mismatches	4	1
1 mismatch	11	5
2 mismatches	3	2
Highly sensitised	5	5
Second or subsequent graft	6	1

a dose of 500 mg daily for the first 3 postoperative days. All patients received 750 mg cefuroxime intravenously immediately before surgery to prevent peri-operative infection and oral cotrimoxazole at a dose of 480 mg once daily for 6 months after transplantation as prophylaxis against *Pneumocystis carinii* pneumonia.

Rejection was diagnosed by standard clinical and laboratory criteria, namely, fever, swelling and tenderness of the graft, and a persistent rise in plasma creatinine. In all cases rejection was confirmed histologically. Treatment in the first instance was with 500 mg intravenous methylprednisolone daily for 3-6 days. Rejection was defined as steroid resistant if there was no response to treatment within 5 days of starting methylprednisolone, or if the creatinine level rose progressively during the first 3 days of methylprednisolone treatment. ATG (Fresenius, Munich, FRG) was given at a dose of 2-3 mg/kg per day as a 4-h slow infusion for 5-10 days. The dose of ATG was reduced to 1-2 mg/kg per day if there was significant leucopenia or thrombocytopenia. During ATG treatment the cyclosporin dose was reduced, or in some patients stopped completely, and started again at the end of the course of ATG. All patients were admitted to hospital during ATG treatment. The response to ATG was defined as successful if the graft was functioning at the most recent follow-up (range 11-38 months from time of transplantation).

The results of examination by light microscopy of sections from paraffin embedded renal biopsies were reviewed. The presence of various features of rejection were noted, using histological criteria previously described by the pathologist [6]. It should be noted that in our unit biopsies are performed routinely each week and at other times for clinical indications. Thus the diagnosis of rejection and its course were documented in a very exact manner.

Statistical analysis was performed by chi squared tests, paired t-tests, Mann Whitney tests, or Pearson's correlation coefficient as appropriate [15].

Results

Details of the numbers of patients who were treated for rejection are shown in Table 1. Two of the grafts that failed after ATG treatment were lost for reasons other than rejection: in one case due to death of the patient, and in another case due to rejection of only moderate severity and subsequent renal artery thrombosis. However, as ATG was given to these two patients with the intention to treat steroid resistant rejection, these patients have not been excluded from further analysis.

Graft survival

The overall graft survival at the time of most recent follow-up in the 27 patients given ATG was 70.3%, with a mean follow-up time of 13.3 months (range 2-30 months). Eight grafts were lost, six as a result of irreversible rejection, one due to patient death and one to renal artery thrombosis.

The mean plasma creatinine level at the start of ATG treatment was 571 μmol/l (range 175-1174), and the mean total dose of intravenous methylprednisolone given before treatment with ATG was started was 2.8 grams per patient. There were no differences between the responders and non-responders to ATG that would have predicted the outcome of treatment (Table 2). Mean plasma creatinine levels at 1 year after transplantation were 151.6 (SEM 16.1) μmol/l in patients following successful ATG treatment, 148.1 (SEM 8.1) μmol/l in all other patients given triple therapy who had not been treated for rejection and 165.0 (SEM 7.5) μmol/l in triple therapy patients with steroid responsive rejection. Eleven of the 19 patients who were successfully treated with ATG required subsequent treatment for rejection with methylprednisolone.

Eight patients who lost grafts due to acute irreversible rejection were not given ATG. The decision not to treat these steroid resistant rejections with ATG was due to concurrent pneumonia ($n=1$), no blood supply on isotope renogram ($n=1$), graft rupture ($n=1$), accelerated rejection with no postoperative function ($n=3$) and patients with previous failed grafts who had already received large doses of immunosuppression ($n=2$).

Renal histology

Renal biopsies were reviewed in all patients. In one case medulla only was obtained on biopsy, but this showed interstitial oedema, diffuse cellular infiltration and interstitial haemorrhage, confirming the diagnosis of rejection. In three other biopsies no arterial vessels of any size were seen.

All biopsies showed cellular rejection, and severe cellular rejection (defined as dense cellular infiltrate with blast cells, and cellular penetration of tubules with some tubular necrosis) was seen both in patients who responded to ATG and in those who did not. Other features of rejection were noted if present: vascular changes, glomerular changes (either capillary thrombosis or cellular infiltration), interstitial haemorrhage and infarction. All of these features were seen in some patients who responded to ATG treatment. Table 3 shows the numbers of patients who had cellular rejection only, or cellular rejection with one or more of these additional features. Vascular changes were seen on biopsy in 11 patients who responded to ATG and included cellular infiltration with intimal oedema and complete luminal obliteration. Interstitial haemorrhage was seen in 5 patients who responded to ATG. However, fibrinoid necrosis of an arteriole and frank glomerular thrombosis were only seen in one case, where there was no subsequent recovery of function.

Patient survival

Two patients died following ATG treatment. The first developed a fever after completing a 10-day course of ATG. There was increased shadowing on his chest radiograph, but examination of bronchoalveolar lavage was negative. He died of respiratory failure 5 weeks after transplantation, and autopsy showed multiple pulmonary emboli and longstanding pulmonary fibrosis, without any apparent infection. The second patient presented with acute rejection 9 months after transplantation. She became febrile a few hours after receiving one dose of ATG and died within 36 h from *Pseudomonas* septicaemia with circulatory failure. A focus of infection was not identified.

Duration of treatment

The duration of treatment in each case was determined partly by the response to treatment and partly by an intention to use a 10-day course as standard therapy. Graft survival and the incidence of adverse effects were not related to duration of treatment. In three cases ATG was given for less than 5 days: in one case because of the death of the patient, in one case because of severe neutropenia, and in one case because of vascular thrombosis of the graft. Treatment with ATG was started during the first month after transplantation in 23 patients, between 1 and 2 months after transplantation in 3 patients, and 9 months after transplantation in 1 patient.

Table 3. Renal biopsy appearances in patients successfully or unsuccessfully treated with ATG for steroid resistant rejection, according to the presence of cellular rejection (CR) plus one or more other features, namely, vascular changes, glomerular changes, interstitial haemorrhage or focal infarction

Histological appearance	Number of responders	Number of non-responders
CR alone	5	3
CR + 1 other feature	10	2
CR + 2 other features	4	1
CR + 3 other features	0	2

Table 4. Infections associated with 27 courses of ATG treatment. There was also 1 pneumonia, causative organism not identified

Infection	Type	Number
Bacterial	*Pseudomonas* septicaemia	1
	Streptococcus faecalis septicaemia	1
	Urinary tract infection	6
	Pseudomembranous colitis	1
Viral	Cytomegalovirus	8
	Herpes simplex	5

Table 5. Changes in haematological indices (mean and SEM) during treatment with ATG. The values immediately before the start of treatment are compared with the lowest value in the next 14 days

	Value on starting ATG	Lowest value	*P*	Mean percentage fall
Haemoglobin (g/dl)	7.5 (0.2)	5.6 (0.2)	< 0.0001	24.3 (2.6)
White cell count ($\times 10^9$/l)	11.1 (4.1)	4.0 (1.9)	< 0.0001	62.2 (16.0)
Platelets ($\times 10^9$/l)	280 (113)	150 (65)	< 0.0001	45.1 (15.0)

Adverse effects

Details of infections occurring within 2 months of treatment with ATG are shown in Table 4. One *Pseudomonas* septicaemia described above was rapidly fatal, but the other case of septicaemia (caused by *Streptococcus faecalis*) was successfully treated with

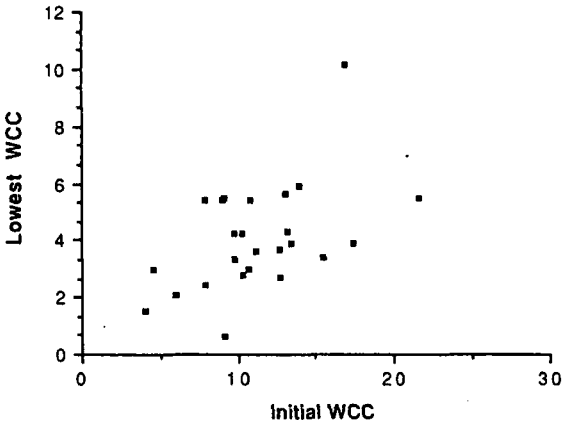

Fig. 1. Peripheral white cell count immediately before the start of ATG related to the lowest value recorded in the next 14 days. White cell count $\times 10^9/1$. $r=0.494$; $P<0.01$

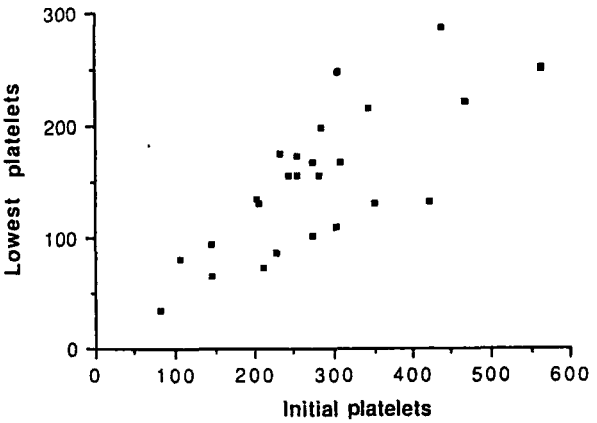

Fig. 2. Platelet count immediately before the start of ATG related to the lowest value recorded in the next 14 days. Platelet count $\times 10^9/1$. $r=0.761$; $P<0.001$

no serious residual morbidity. Cytomegalovirus (CMV) infection was diagnosed either by serological evidence of infection (appearance of IgM antibody in the serum) or by isolation of the virus. Only one patient with CMV infection had a serious illness, with fever and pneumonitis developing during ATG treatment. Withdrawal of immunosuppression and graft nephrectomy were followed by a gradual recovery.

There were significant falls in haemoglobin concentration, white cell count and platelet count during ATG therapy (Table 5). The magnitude of the falls in white cell count and platelet count were related to the level on the first day of treatment ($r=0.885$, $P<0.001$; $r=0.842$, $P<0.001$, respectively; Figs. 1, 2). There was a less strong relationship between haemoglobin concentration on the first day of treatment and the fall observed ($r=0.58$, $P<0.01$). The mean time from the start of ATG treatment to the lowest haemoglobin concentration was 8.5 (SEM 0.7) days, to the lowest white cell count 7.8 (SEM 0.7) days and to the lowest platelet count 5.6 (SEM 0.6) days. The fall in platelet count occurred earlier than the fall in haemoglobin or white cell count ($P<0.01$ and $P<0.05$, respectively).

None of our patients treated with ATG has developed malignancies of any sort, but follow-up is very short.

HLA matching and immunologically high-risk patients

Details of the matching for HLA DR antigens in the patients given ATG are shown in Table 1. (In one case it was not possible to determine the DR type of the donor due to lack of suitable lymphoid tissue.) There was a tendency for steroid resistant rejection to occur more often in patients with DR mismatches than in those without DR mismatches, but this difference was not statistically significant: 5 of 53 patients (9.4%) with no DR mismatches experienced steroid resistant rejection, compared to 21 of 130 (16.4%) with 1 or 2 DR mismatches. DR matching did not influence the outcome of steroid resistant rejection.

Forty-six of the 187 grafts were performed in immunologically high-risk patients (as defined in "Methods"). The incidence or outcome of steroid resistant rejection treated with ATG was not related to either of these factors in this series.

Discussion

In this series 70% of steroid resistant rejection episodes in patients receiving triple therapy were reversed, a success rate for ATG treatment of resistant rejection similar to that seen in patients receiving only prednisolone and azathioprine, but rather better than that observed in reported series of patients receiving cyclosporin and prednisolone [3, 4, 13, 14]. All our patients treated with ATG had the diagnosis of severe rejection confirmed by renal biopsy, and the fact that ten of our patients required dialysis for oliguria suggests that as a group our patients were suffering from severe rejection. The potential risks of ATG administration in these patients might be difficult to justify if the outcome was frequently an allograft that was badly scarred with subsequent failure in the medium term. However, the outcome in this series was generally good, both in terms of immediate graft survival and medium term graft function.

The incidence of steroid resistant rejection treated with ATG in our series is 14.4%; in addition, eight patients with resistant rejection were not given ATG, giving a total incidence of 18.7%. Despite this appreciable incidence of serious rejection, we feel that triple therapy is an effective regimen in our patients. Nearly one-third of our patients experience no rejection episodes, and we do not feel that the risks of administering additional immunosuppression, for example the use of prophylactic ATG in a quadruple therapy regimen, would be justified.

There were no obvious clinical or histological features of severe rejection that would enable the identification of patients most likely to benefit from ATG therapy. In particular, histological changes classically regarded as indicative of severe and often irreversible rejection, such as vascular rejection with arterial obliteration, glomerular infiltration by lymphocytes, interstitial haemorrhage and focal infarction, were all seen in patients whose grafts recovered after treatment. However, the combination of cellular rejection and at least three of these other features of severe rejection was seen only in two patients, in both of whom there was subsequent graft failure.

There is a potential risk of over-immunosuppression in patients treated with triple therapy and ATG, as serious morbidity and mortality have been reported in patients treated with ATG and cyclosporin [8, 14]. Triple therapy alone is not associated with a high incidence of serious infection in our experience [12], but it has seemed prudent to reduce the dosage of cyclosporin during concurrent ATG administration. Even so, there was a significant incidence of infection and mortality in this series, although the relationship between the two deaths and the ATG treatment is not entirely clear. Nevertheless, we feel that the use of ATG is justified, as the risk of serious infection during treatment is more than offset by the salvage of the majority of kidneys undergoing severe steroid resistant rejection and the good renal function achieved in the medium term in those kidneys responding to therapy.

Falls in white cell count, platelet count and haemoglobin concentration were seen in all patients and were related closely to the levels on the first day of ATG treatment. It should, therefore, be possible to identify patients at risk of severe leucopenia or thrombocytopenia before treatment is begun and so to consider early withdrawal of azathioprine. We have previously shown that cotrimoxazole administered to prevent *Pneumocystis carinii* pneumonia increases the risk of leucopenia in patients receiving triple therapy [9], and it is possible that it contributed to the leucopenia seen after ATG treatment in this series. In patients who are at high risk of developing leucopenia during ATG therapy, it may be desirable to give cotrimoxazole on just 4 consecutive days a week, as it has been shown that this regimen provides effective protection against *Pneumocystis* pneumonia [10]. The consistency of the haematological effects over the 3-year period of this study also suggests that there has been little batch-to-batch variation in the preparation of the ATG used.

Monoclonal antibody therapy has recently been introduced into clinical practice, and the antibody OKT3 (Ortho Corporation, Raritan, NJ, USA) has now been used in many patients with steroid resistant rejection [5, 17, 18, 21]. The specificity of OKT3 for T cells in theory offers some advantages, as does the consistency of the properties of such a monoclonal antibody. However, we are impressed by the fact that the rabbit ATG used throughout our own series and reported here has appeared entirely consistent in its immunosuppressive potency. In patients immunosuppressed with cyclosporin and steroids, or azathioprine and steroids, OKT3 has reversed 80%-90% of resistant rejection episodes in published series and has been associated with a similarly high incidence of infections (e.g. one death from CMV infection [21], one case of *Listeria* septicaemia [5], and a 25% incidence of oropharyngeal herpes simplex [21]). It has been suggested in one small series that OKT3 may be particularly effective in treating steroid resistant rejection with predominantly vascular changes on renal biopsy [5]; the present study suggests that ATG may also be effective in treating this form of rejection. In the absence of any randomised controlled trials, there is no evidence as yet to suggest that OKT3 is preferable to ATG in the treatment of severe rejection.

In conclusion, this study has shown that steroid resistant rejection is a significant problem in patients immunosuppressed with triple therapy and that 70% of such rejections can be reversed following ATG treatment. Dose reduction or withdrawal of cyclosporin during ATG therapy may help avoid over-immunosuppression and serious opportunistic infections. Those patients at most risk of developing severe leucopenia or thrombocytopenia can be identified before treatment is started. The outcome for renal allografts after ATG responsive rejection appears to be excellent for at least the first 2 years of follow-up, even in those patients who required dialysis for acute renal failure associated with the steroid resistant rejection.

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