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Administration of ATG according to the absolute T lymphocyte count during therapy for steroid-resistant rejection

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Abstract. In renal transplantation, treatment of steroid-resistant rejection (SRR) with antithymocyte globulin (ATG) has been widely reported but over-immunosuppression remains a common problem. In the first ten patients (group 1) treated for SRR with rabbit ATG, three developed serious viral infections and two deaths occurred due to CMV pneumonitis. ATG was only omitted if thrombocytopenia or neutropenia occurred. In the next 17 patients (group 2) with SRR, ATG was administered according to the absolute T lymphocyte count. T lymphocytes were measured by flow cytometric analysis of CD3-labelled lymphocytes. ATG dosage was adjusted on a daily basis to keep the absolute T lymphocyte count under 50 cells/μl. Administration of ATG according to the absolute T lymphocyte count resulted in a significant reduction in the mean dose of ATG given to the group 2 patients ($P < 0.001$). A significant decrease in the incidence of serious viral infections ($P = 0.04$) was achieved without reducing the ability of ATG to reverse the SRR ($P = 0.29$) or increasing the number of grafts lost at 1 year in the group 2 patients ($P = 0.23$).

Key words: Kidney transplantation, rejection, ATG – ATG, rejection, kidney transplantation – T lymphocyte count, ATG, kidney rejection

Antilymphocyte globulin (ALG) was first introduced to clinical renal transplantation in 1967 [10]. Early clinical studies involved the prophylactic use of ALG from the time of transplantation. It was not until 1979 [9] that reversal of acute renal allograft rejection with ALG as the only therapy was recognised. In the same year, the first report of the use of antithymocyte globulin (ATG) in the treatment of steroid-resistant rejection (SRR) was published [4]. Since then the use of ATG for the treatment of SRR has become widely established. One-year graft survival for patients who have been treated with ATG for SRR ranges from 55% to 75% [1, 11]. These studies have confirmed ATG as a potent immunosuppressive agent, but variation of potency does occur between ATG produced from different animal sources and even between different batches of the same product. In addition, patients respond in an idiosyncratic way when given ATG. Thus, when ATG is administered according to a fixed dose regimen, these two factors may result in over- or underimmunosuppression of the patient. Over-immunosuppression usually manifests itself as infection, in particular viral infections, such as cytomegalovirus (CMV). Most studies of the use of ATG for the treatment of SRR report appreciable patient morbidity and even mortality from infectious complications [5, 6, 8]. In order to reduce the risk of over-immunosuppression, we initially investigated whether the absolute lymphocyte count or the differential lymphocyte count was a better way to monitor ATG therapy than the white cell count (WCC) and the platelet count that the manufacturers recommended. Following this we developed a technique that requires daily monitoring of patients during ATG administration with daily adjustment of the dose of ATG according to the absolute T lymphocyte count.

Materials and methods

Between January 1988 and December 1990, 367 patients received renal allografts in our centre. Of these, 27 patients experienced SRR, which was treated with rabbit ATG (Institut Merieux, Lyon, France). The first 10 patients (group 1) were treated with ATG administered according to the manufacturer's recommendations. The remaining 17 patients (group 2) were treated with ATG administered according to the absolute T cell count.

All patients received 10 mg/kg of cyclosporin A (CyA) following renal transplantation. Recipients of first grafts received CyA monotherapy, whereas recipients of second grafts received 25 mg of oral prednisolone in addition to CyA. Initially, rejection episodes were treated with intravenous methylprednisolone. All of the patients in group 1 received 500 mg of methylprednisolone daily for 3 days, as did ten patients in group 2. The remaining seven patients in group 2 received 3 mg/kg of methylprednisolone daily for 3 days, followed by 500 mg of methylprednisolone for 3 days if the patient

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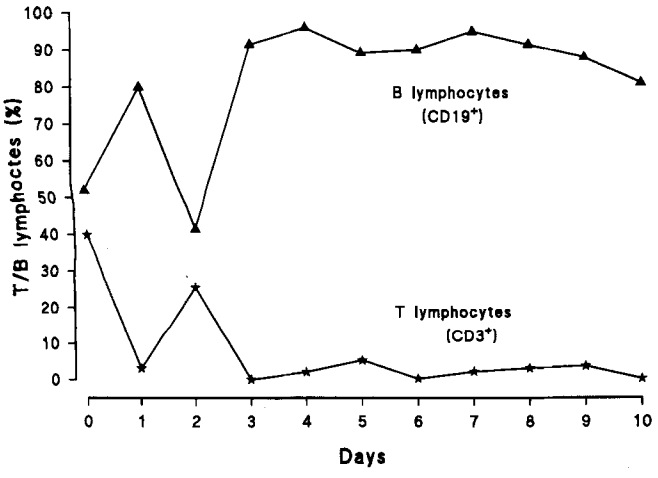

Fig. 1. The percentage of T and B lymphocytes measured on a daily basis from a patient receiving ATG for SRR over a 10-day period

failed to respond. All patients whose rejection episodes failed to respond to methylprednisolone or in whom rejection quickly recurred were considered to have "steroid-resistant" rejection. Continuing rejection was confirmed by a percutaneous renal core biopsy in all patients prior to starting ATG therapy.

To investigate whether the differential lymphocyte count or the absolute lymphocyte count was a better way in which to monitor ATG therapy, we studied the phenotype of lymphocytes during ATG therapy for SRR in the last three patients in group 1. Ten millilitres of heparinised blood was venesected daily. This was diluted 1:1 with Earle's balanced salt solution (Northumbria Biologicals, UK) and centrifuged for 25 min at 400 *g* over a Ficoll-Metrizoate (Lymphoprep, Nycomed, UK) density gradient. The interfacial band of mononuclear cells was removed and washed once with Isoton II (Coulter Electronic, UK) and centrifuged at 300 *g* for 10 min. The percentage of T lymphocytes was calculated using a fluoroscein isothiocyanate (FITC)-conjugated anti-CD3 monoclonal antibody and the percentage of B lymphocytes using a FITC-conjugated anti-CD19 monoclonal antibody (Becton Dickinson, Oxford, UK). Two microlitres of the monoclonal antibody was added to the peripheral blood mononuclear cells and resuspended with 10 µl of Isoton II prior to incubation at 4 °C for 20 min. The cells were then washed with Isoton II and centrifuged for 10 min at 2874 *g*. This step was repeated twice. The final cell pellet was resuspended with 500 µl of Isoton II and analysed using a FACScan flow cytometer with Lysis II computer software (Becton Dickinson, Oxford, UK). Lymphocyte gating on side and forward lazer scatter was optimised by double staining lymphocytes with phycoerythrin-conjugated anti-human Leu-M3 (CD14) and FITC-conjugated anti-human leucocyte (CD45). We aimed for a lymphocyte purity of over 70%. When the lymphocyte purity was less than 70%, we reported that few lymphocytes were present.

Administration of ATG in group 1 and group 2 was as follows: In group 1, ATG was administered at a dosage of 2.5–5 mg/kg per day. According to the manufacturer's recommendations the dosage was reduced or omitted if thrombocytopenia (platelet count $< 50 \times 10^9$/l) or leucopenia (white cell count $< 4 \times 10^9$/l) occurred. In group 2, ATG was administered according to the absolute T lymphocyte count. This was calculated on a daily basis from the percentage of T lymphocytes multiplied by the absolute lymphocyte count. The percentage of T lymphocytes was measured by flow cytometric analysis of CD3-positive lymphocytes, as described previously. The absolute lymphocyte count was measured from a coulter counter analysis of the same sample of blood. The aim was to keep the absolute T lymphocyte count under 50 cells/µl. If the absolute T lymphocyte count was greater than 50 cells/µl, ATG was given for that day. However, if the absolute T lymphocyte count was under 50 cells/µl, ATG was omitted for that day. During ATG therapy the patients received 25 mg of oral prednisolone daily and the CyA was stopped. The duration of the ATG course was determined by the patient's clinical and biochemical response over the first 4–5 days of therapy and ranged from 10 to 14 days. Three days before the ATG course was due to finish, oral CyA at a dose of 6 mg/kg and 1 mg/kg of oral azathioprine was commenced. The dose of CyA was adjusted to keep the whole blood trough levels between 200 and 400 ng/ml. Following therapy with ATG, patients were started on triple immunosuppression of CyA, prednisolone and azathioprine. The prednisolone was tapered to a maintenance dose of 10 mg/day.

In both group 1 and group 2, patients received cotrimoxazole for prophylaxis against *Pneumocystis carinii* during ATG therapy. In our centre CMV prophylaxis is not given to all patients during ATG therapy. However, CMV prophylaxis was given from the time of transplantation in one patient from group 1 who was CMV IgG-negative and who received a kidney from a CMV IgG-positive donor.

Statistical analysis of results, comparing group 1 with group 2, was performed using an unpaired *t*-test or a Fischer's exact test, as appropriate.

Results

Figure 1 shows the daily composition of the lymphocyte population in a patient receiving AGT for SRR over a 10-day period. It can be seen that particularly after 3 days of ATG, the main population of lymphocytes present consists of B lymphocytes and not T lymphocytes, the primary target for ATG.

The mean number of rejection episodes experienced by group 1 and group 2 patients prior to the commencement of ATG was 1.5 and 1.8, respectively. This difference was not significant ($P = 0.33$). There was also no significant difference in the mean cumulative dose of methylprednisolone given before ATG was commenced ($P = 0.52$). Group 1 patients received 3.9 g and group 2 patients 3.6 g of methylprednisolone.

Although a historical group (group 1) was used for comparison with the experimental group (group 2), when the two groups were compared for the major variables that affect renal allograft outcome, there was no signifi-

Table 1. Comparison of group 1 with group 2. Pts, Number of patients

Variable		Group 1 ($n = 10$)		Group 2 ($n = 17$)		
Graft no. (Pts)		1st	9	1st	12	NS
		2nd	1	2nd	5	
Sex		F	3	F	4	NS
		M	7	M	13	
Mean age (years)			42.6		43.2	NS
Peak cytotoxic antibodies (%)			23.0		13.8	NS
Mean HLA B mismatch			0.9		0.6	NS
Mean HLA DR mismatch			1.1		0.8	NS
Delayed graft function (Pts)			2		5	NS
Pre-ATG biopsy result (Pts)						
Cellular	Mild		3		5	
	Moderate/severe		7		12	NS
Vascular	Present		6		11	
	Not present		4		6	NS

Table 2. Group 1 and group 2 patients following ATG therapy. Pts, Number of patients

	Group 1 ($n = 10$)	Group 2 ($n = 17$)
Mean no. of days in hospital	44.6	37.8
Length of ATG course (days)	10.0	11.4
Incomplete courses of ATG (Pts)	3	0
Mean dosage of ATG administered (mg/kg per day)	2.2	0.9
Serious viral infection (Pts)	3	0
Patient deaths due to infection	2	0
Minor infections (Pts)	6	11
Patients mean WCC ($x10^9/l$)	6.5	8.9
Patients mean platelet count ($x10^9/l$)	167	215
Reversal of SRR (Pts)	8	16
Rejection episodes after ATG (no. episodes/patient)	0.8	0.35

cant difference (Table 1). In particular, there was no significant difference in the severity of rejection seen on histological examination of the pre-ATG core biopsy.

Table 2 shows that administration of ATG according to the absolute T lymphocyte count in the group 2 patients did not result in a longer hospital stay ($P = 0.22$) or a shorter course of ATG being administered ($P = 0.15$) when compared with group 1 patients. However, 3 patients in group 1 had their course of ATG stopped early. One patient developed systemic CMV infection and 2 patients became leucopenic. None of the 17 patients in group 2 had to have his course of ATG stopped early. In group 2, ATG was not given every day, but the interrupted course of ATG was by design and not due to the complications of ATG therapy.

The first significant factor we noticed was that the mean dose of ATG administered to group 2 patients was less than the mean dose administered to group 1 patients (Table 2). The difference was strongly significant ($P < 0.001$). There was also a significant reduction in the incidence of serious viral infections in group 2. Three patients in group 1 developed a serious viral infection with two deaths from CMV pneumonitis, whereas no patient in group 2 developed a serious viral infection ($P = 0.04$). There was, however, no significant difference between group 1 and group 2 for the incidence of minor infections, e. g. herpes simplex labialis, oral candida or a urinary tract infection ($P = 0.21$).

Administration of less ATG enabled patients in group 2 to maintain their WCC and platelet counts (Table 2) at a higher level than group 1 patients. The difference was significant for the WCC ($P = 0.04$) but not for the platelet count ($P = 0.09$). The reduction in dosage of ATG administered to group 2 patients reduced the complications of over-immunosuppression, but we were concerned that we might also reduce the efficacy of our therapy.

There was no significant difference in the ability of ATG to reverse the SRR episodes ($P = 0.26$) in group 2 patients compared with group 1 patients (Table 2). For patients who had successful reversal of SRR, there was no significant difference between group 1 and group 2 for the occurrence of further rejection episodes ($P = 0.13$) (Table 2). Most importantly, the reduction in the dose of ATG given to group 2 patients did not affect the 1-year graft survival (Table 3). The four grafts lost in group 1 patients were all due to rejection. However, in the group 2 patients, one graft was lost to rejection, one graft never functioned following transplantation, one patient died of an unrelated cause and one patient refused further treatment when a rejection episode occurred 3 months after successful reversal of the SRR with ATG. Assessment of graft function at 1 year post-transplantation (Table 3), as measured by serum creatinine, revealed no significant difference between group 1 and group 2 ($P = 0.74$).

Discussion

Despite 20 years' clinical experience with ATG, the complications of over-immunosuppression remain a major concern to any clinician using this potent immunosuppressive agent. In the first ten patients, in whom we used ATG for the treatment of SRR, two patients (20%) died from CMV pneumonitis. ATG had been administered according to the manufacturer's recommendations with the dose of ATG being omitted or reduced if thrombocytopenia or leucopenia occurred. We felt that administration of ATG according to the WCC and platelet count was an unsatisfactory way in which to monitor therapy. Monitoring the WCC and platelet count does not reflect the clinical effect of ATG and it does not avoid the complications of infection. As Fig. 1 shows, monitoring the absolute lymphocyte count or the differential lymphocyte count mainly monitors B lymphocytes and not the T lymphocytes against which ATG is primarily directed. We therefore decided to monitor the T lymphocytes daily with adjustment of the ATG therapy according to the results.

Monitoring of the circulating T lymphocytes during ATG therapy is not a new concept. Cosimi et al. [2] reported increased renal allograft survival in patients treated prophylactically with ATG if the dose of ATG was adjusted to keep the number of lymphocytes rosetting with sheep red blood cells (E-rosette assay: an indirect measure of the circulating T lymphocytes) less than 10% of the pretreatment value. However, Thomas et al. [12] suggested that the optimal mode of T cell and T cell subset monitoring in patients receiving ATG was by using monoclonal antibodies, as the E-rosette assay was unreliable and inaccurate. This was confirmed in a study by Ganghoff et al. [3]. Thomas et al. [12] also reported an association between rejection episodes and T cell levels increasing above 100 cells/μl on at least three occasions in the week prior to the rejection episode. Following this, Williams et al. [13] reported increased graft and patient survival with a decreased incidence of rejection episodes if the total circulating T cell levels were kept under 100 cells/μl. He concluded that measurement of the T cell levels by flow cytometry and monoclonal antibodies was accurate, reproducible and rapidly obtained. However, monitoring

Table 3. 1-Year follow-up of patients treated with ATG for SRR. Pts, Number of patients

	Group 1 ($n = 10$)	Group 2 ($n = 17$)
Graft survival at 1 year (pts)	6 (60%)	13 (76%)
Mean serum creatinine at 1 year (nmol/l)	250	229
Mean cyclosporin dose at 1 year (mg/kg per day)	5.45	5.15

was only performed three times per week and over a 90-day period. We monitored our patients daily, with adjustment of the dose of ATG to keep the absolute T cell count under 50 cells/μl. This was only for the 12-day period during which ATG was administered.

The incidence of CMV infections in patients receiving ATG for the treatment of SRR varies from nil [1] to 29% [8]. Not all of these patients are symptomatic, but mortality from infection following ATG therapy is commonly reported [5, 8, 11].

Pass et al. [7], in 1980, reported a direct correlation between the development of CMV infection and the dosage of ATG used. Therefore, although the number of patients in our study was small, the reduction in dosage of ATG in the group 2 patients was a significant factor.

Other studies have found the occurrence of minor infections during ATG therapy to be common [6, 11]. We found that although they cause a degree of patient morbidity, they are readily treated with appropriate therapy.

None of the patients in group 2 had their course of ATG shortened due to complications of ATG therapy. Febrile reactions are commonly seen with ATG therapy, particularly with the first two doses, but these are easily controlled with prophylactic hydrocortisone and chlorpheniramine. Stopping the course of ATG for whatever reason has a significant effect on the ability of ATG to reverse the SRR. Matas et al. [5] and O'Donoghue et al. [6] both reported poor graft outcome in those patients who had an incomplete course of ATG.

During this study we felt our main achievement was the reduction in dosage of ATG by greater than one-half without loss of clinical efficacy. Reversal of the SRR with ATG in group 2 patients occurred in 94% of the patients. This is comparable with other studies reporting 52% [5], 80% [1] and 70% [8]. Our 1-year graft survival of 76% in group 2 is also comparable with previous studies [1, 6, 8, 11].

Finally, reducing the dosage of ATG administered for the treatment of SRR results in cost savings. In our unit the cost for 12 days of flow cytometric monitoring is approximately £180. Reducing the dose of ATG administered by an average of more than 1 mg/kg per day, as we achieved, results in a saving of £1620 in the cost of ATG. This represents a net saving of £1440 per patient.

Monitoring of ATG during therapy for SRR, according to the absolute T lymphocyte count, has resulted in administration of less ATG. The reduced dose of ATG has achieved a significant reduction in the incidence of serious viral infections. However, the reduced dose of ATG has not altered the ability of ATG to reverse the SRR episode, nor has it resulted in an increase in the number of grafts lost at 1 year. Although we used a historical group for comparison with the treatment group and the overall numbers studied were low, we are not going to perform a prospective, randomized study due to the small number of patients we have with SRR each year and due to the clear cost effectiveness of administering ATG according to the absolute T lymphocyte count.

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