Nephrol Dial Transplant (1998) 13: 711–715

Nephrology
Dialysis
Transplantation

Original Article

Advantage of antithymocyte globulin induction in sensitized kidney recipients: a randomized prospective study comparing induction with and without antithymocyte globulin

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Abstract

Background. Sensitized kidney allograft recipients require special management to improve their outcome. One strategy is heavy immunosuppression with antilymphocyte antibodies. Controversy continues about the actual advantage of induction protocols whilst infections and cancers are a constant risk. In addition, little is known about how to handle sensitized patients with low levels of sensitization.

Methods. In this study, we randomized sensitized renal transplant recipients, who received prophylactic treatment with or without antithymocyte globulin (ATG), in addition to a standard triple regimen consisting of cyclosporin, steroids and azathioprine at ATG discontinuation. The induction treatment consisted of a low-dose ATG course over 10 days. Randomization was stratified on the maximum PRA, according to the five following classes: $5\% < PRA \leqslant 20\%$, $20\% < PRA \leqslant 40\%$, $40\% < PRA \leqslant 60\%$, $60\% < PRA \leqslant 80\%$ and $80\% < PRA \leqslant 100\%$.

Results. Eighty nine patients were enrolled: 47 patients received ATG and 42 did not. ATG induction lowered the incidence of biopsy-proven acute rejection episodes from 64 to 38%, increased 1 year graft survival from 76 to 89% and was associated with a higher 1 year inulin clearance (37 ± 15 *vs* 49 ± 18 ml/min). ATG-associated side effects were restricted to leucopenia and thrombocytopenia, whereas bacterial and viral infections, gammopathies and cancers did not occur more frequently. ATG induction benefited all sensitized patients, and not only the hypersensitized patients.

Conclusions. We conclude that ATG induction is beneficial for all sensitized patients, regardless of their level of sensitization, with regard to acute rejection episodes, graft survival and graft function. Low-dose ATG is sufficient and prevents additional complications.

Key words: antithymocyte globulin; immunosuppression; induction therapy; renal transplantation; triple therapy

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Introduction

Patients awaiting renal transplantation who have lymphocytotoxic antibodies are known to be at a high risk of encountering several difficulties. These include a positive crossmatch, a primary non-functioning allograft, an acute rejection episode and, in the long-term, a reduced graft survival [1–3]. Many strategies have been designed to manage sensitized recipients; among these, the use of a heavy prophylactic immunosuppression has been proposed, adding antilymphocyte globulin (ALG), antithymocyte globulin (ATG) or anti-CD3 monoclonal antibodies (OKT3), in what are named induction therapies. Although heavy immunosuppression has been proved to be effective [4], the actual advantage of these induction therapies may be questioned with regard to some other studies which have reported only a very modest effect on overall graft survival [5]. Protocols with antilymphocyte antibodies have been designed mainly to delay the introduction of cyclosporin and its associated nephrotoxicity [6–8]. Patients suffering from delayed graft function may represent a subgroup which would benefit from sequential therapies [9]. Heavy immunosuppressive protocols with antilymphocyte antibodies and cyclosporin given simultaneously have already been proposed [10], but not always in comparative studies [11]. Most of the comparisons were based on the prophylactic use of ALG and OKT3, neither of which has proved to be superior. Finally, information on sensitized patients remains scarce [12].There is no evidence to suggest that highly sensitized patients require more prophylactic immunosuppression than the less sensitized patients and, moreover, at present we do not know the effect of induction therapies in sensitized, but not highly sensitized patients [13].

In our own experience with sensitized recipients, the

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best graft survival rate was achieved with the early introduction of cyclosporin, even when compared with sequential induction with antilymphocyte antibodies. We wanted to analyse the advantage that an induction therapy could add to cyclosporin usage in sensitized patients. We therefore conducted a PRA-stratified, randomized prospective study to compare induction with and without ATG in sensitized cyclosporin-treated kidney recipients.

Subjects and methods

Patient population

Conducted from 1991 to 1995, this study included all sensitized patients receiving either a first or a second graft, from a cadaveric or a living donor, even in the case of a historical or current positive B-cell crossmatch. Third grafts and grafts performed against a positive historical T-cell crossmatch were excluded. Sensitization was defined by the presence in the patient's serum of lymphocytotoxic antibodies recognizing at least 5% of a panel of 24–30 whole lymphocytes from different donors. The randomization was stratified on maximum PRA in order to obtain a well-balanced distribution, whatever the level of sensitization. We defined the following five classes: $5\% < PRA \leqslant 20\%$, $20\% < PRA \leqslant 40\%$, $40\% < PRA \leqslant 60\%$, $60\% < PRA \leqslant 80\%$ and $80\% < PRA \leqslant 100\%$. This last class refers to the classic definition of hypersensitized patients.

Immunosuppressive protocols

The triple therapy consisted of cyclosporin, azathioprine and steroids. Cyclosporine (Sandimmum®, Sandoz) was introduced before graft implantation, at an oral equivalent of 14 mg/kg/day dose, tapered every second day to obtain an 8 mg/kg/day dose at the end of the first week. Further adjustments were made carefully according to daily trough levels, with a 100–300 μg/l target range. Azathioprine was given at 2 mg/kg/day and prednisolone at 30 mg/day. ATG induction comprised the same therapy with cyclosporin and prednisolone, plus a 10 day ATG course (Thymoglobulin®, IMTIX Pasteur-Mérieux-Connaught, Lyon, France), given at a 1.25 mg/kg/day as the starting dose. The number of peripheral CD2 and CD3 lymphocytes was monitored three times a week to adjust ATG doses. Azathioprine was introduced at ATG discontinuation. Acute rejection was first treated by high-dose pulse steroids, then with OKT3 in cases of unresponsiveness.

Study endpoints

The main criterion of efficacy was the occurrence of an acute rejection episode. The clinical diagnosis of acute rejection was made in cases of delayed graft function or in cases of a secondary rise in serum creatinine, in the presence of one of the following: urine output < 1 l/24 h, weight gain > 1 kg/24 h, tenderness of the graft or low sodium excretion. Colour duplex sonography, isotopic nephrography, whole blood trough and peak cyclosporin A levels were used to eliminate any other cause of graft dysfunction. Any graft dysfunction which was not explained eventually led to a biopsy. Rejection episodes were confirmed based on the results of a percutaneous 18-gauge needle biopsy. Pathological features were divided into two categories, cellular rejection and vascular rejection. The Banff classification was only applied retrospectively at the end of the study. When rejection was clinically suspected but without histological confirmation, a presumptive rejection episode was considered. Secondary criteria were early side effects, complications, graft survival and graft function. Early side effects were considered within 3 weeks after transplantation, with particular attention to fever and haematological tolerance. A leukocyte count below 3 g/l was considered as leucopenia, a platelet count below 150 g/l as thrombocytopenia. Delayed graft function was defined as the need for dialysis within the first week. Every event occurring within 3 months was considered as a complication. All gammopathies and cancers were recorded. We considered as cytomegalovirus (CMV) infections asymptomatic serological conversions and clinical CMV diseases. Graft survival was calculated according to Kaplan–Meier. Graft function was assessed by 1 year serum creatinine and inulin clearance.

Results

Demography

Forty two patients were given a triple therapy without ATG and 47 received ATG. The actual ATG dose was 0.79 ± 0.19 mg/kg/day (0.5–1.3). On comparison, there was no difference between the two groups with regard to pre-therapeutic conditions and, in particular, immunological status (Table 1). In both groups, the numbers of second grafts, mean PRA, human leukocyte antigen (HLA) mismatch and positive B crossmatches were similar. The patients were equally distributed with regard to PRA stratification, with 12 and 14 patients with $5\% < PRA \leqslant 20\%$, 14 and 12 patients with $20\% < PRA \leqslant 40\%$, five and six patients with $40\% < PRA \leqslant 60\%$, four and five patients with $60\% < PRA \leqslant 80\%$ and finally seven and 10 patients with $80\% < PRA \leqslant 100\%$ respectively.

Acute rejection episodes

Presumptive rejection episodes numbered 30 (71%) in the triple therapy group and 25 (53%) in the ATG

Table 1. Demographics of the patients

	No ATG ($n=42$)	ATG ($n=47$)
Second transplantation	16 (38%)	13 (28%)
Living donor	1	0
Duration of dialysis (months)	66 ± 69	63 ± 64
Females	12 (29%)	19 (40%)
Recipient age	46 ± 13	47 ± 12
Donor age	35 ± 15	39 ± 12
Maximum PRA (%)	42 ± 30	46 ± 32
HLA A/B/DR mismatch	2.4 ± 1.0	2.5 ± 1.0
HLA DR mismatch	0.5 ± 0.6	0.6 ± 0.5
Warm ischaemia (min)	25 ± 9	24 ± 8
Cold ischaemia (h)	29 ± 8	29 ± 9
B cell-positive crossmatch	7 (17%)	13 (28%)

The *P*-value was not significant in all cases.

group ($P=0.008$). There was also a significant difference in the incidence of biopsy-proven acute rejections: 27 (64%) in the triple therapy group and 18 (38%) in the ATG group ($P=0.02$). The mean onset of the first acute rejection episode was 8 ± 8 days in the triple therapy group *vs* 24 ± 45 days in the ATG group ($P=0.02$). The median onset was 8 days (4–31) and 5 days (1–182) respectively. The percentage of biopsies with vascular lesions was similar in the two groups (33 *vs* 39%). Rejection episodes were also analysed according to PRA (Figure 1). The advantage of ATG induction was somewhat different according to the level of sensitization and, surprisingly, highly sensitized patients did not exhibit any greater benefit from ATG induction. When patients were grouped on the basis of the lowest PRA, a clear reduction in rejection episodes appeared in each group (Table 2).

Secondary end points

The use of ATG induced more leucopenia (43 *vs* 17%, $P=0.007$) and thrombocytopenia (32 *vs* 17%, $P=0.008$). Apart from these two early side effects, adverse events were similar in the two groups. We did not note any difference between the triple therapy group and the ATG group with regard to fever (26 *vs* 36%), CMV infection (40 *vs* 59%), Epstein–Barr virus and herpes simplex virus infections (4 *vs* 4%), serious bacterial infections (12 *vs* 13%), urinary tract infections (26 *vs* 24%), gammopathies (12 *vs* 8%) and *de novo* cancers (7 *vs* 2%). Delayed graft function, which may dispose to rejection, occurred in 33% of the patients in the triple therapy group and in 28% of the patients in the ATG group (not significant; NS).

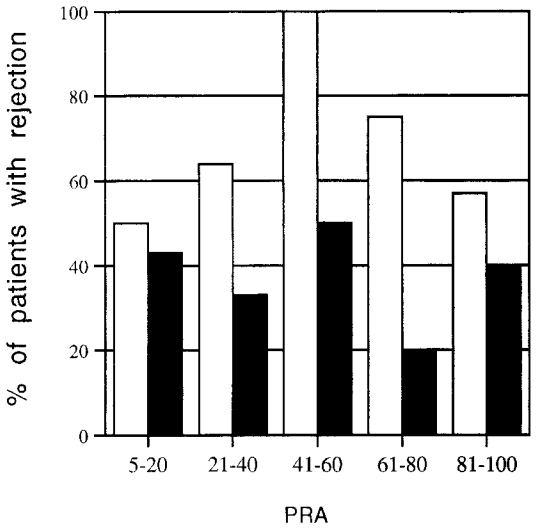

Fig. 1. Percentage of sensitized kidney recipients who experienced a biopsy-proven acute rejection episode with ATG induction (filled bars) or without ATG induction (open bars).

Table 2. Frequency of biopsy-proven acute rejection episodes in patients grouped on the basis of the lowest PRA

PRA	No. of patients	No ATG $n=42$	ATG $n=47$	P
>5%	89	(27/42) 64%	(18/47) 38%	0.02
>20%	63	(21/30) 70%	(12/33) 36%	0.002
>40%	37	(12/16) 75%	(8/21) 38%	0.04
>60%	26	(7/11) 64%	(5/15) 33%	NS
>80%	17	(4/7) 57%	(4/10) 40%	NS

The median duration of follow-up was 25 months, ranging from 1 to 52 months. Graft survival in the triple therapy group and in the ATG group was 81 *vs* 94% at 6 months and 76 *vs* 89% at 12 months respectively (Figure 2) (Breslow: $P=0.06$, Mantel–Cox: $P=0.04$). However, the causes of graft lost were not equally distributed. Non-immunological graft failures occurred mainly in the triple therapy group (6/12), with two surgical failures, one recurrent glomerulonephritis and three deaths, and were less frequent in the ATG group (1/5) with one venous thrombosis. Acute or chronic rejections represented six out of the 12 graft failures in the triple therapy group (50%) and four out of the five graft failures in the ATG group (80%). When the graft survival calculation was censored for non-immunological graft failures, we found a much smaller difference, but still a trend in favour of ATG, with 84 and 91% 1-year graft survival respectively (NS).

Finally, we compared graft function at 1 year. Serum creatinine was 168 ± 77 μmol/l in the triple therapy group *vs* 146 ± 56 μmol/l in the ATG group (NS), but inulin clearance was 37 ± 15 ml/mn *vs* 49 ± 18 ml/mn respectively ($P=0.005$).

Level of sensitization and positive crossmatches

We looked for a possible effect of the level of sensitization on the occurrence of an acute rejection episode as well as on graft survival. Rejection occurred in 50% of patients with $5\%<\text{PRA}\leqslant20\%$, in 77% of patients with $20\%<\text{PRA}\leqslant40\%$, in 73% of patients with $40\%<$

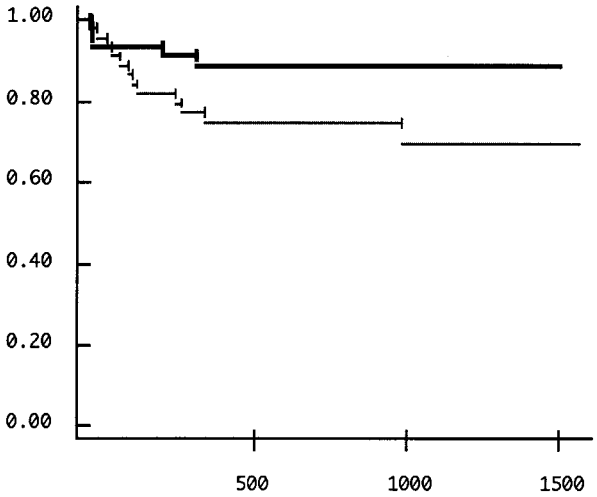

Fig. 2. Graft survival in patients treated with ATG induction (bold line) or without ATG induction (thin line). The *x*-axis represents days.

PRA $\leqslant$ 60%, in 56% of patients with 60% $<$ PRA $\leqslant$ 80% and in 53% of patients with 80% $<$ PRA $\leqslant$ 100% (NS). We found similarly that graft survival was not affected by the level of sensitization (data not shown). The consequences of having a positive crossmatch were also examined. Fifty percent of patients who had either a positive or a negative crossmatch developed an acute rejection. One year graft survival was 81% in patients with a negative crossmatch and 88% in patients with a positive crossmatch (NS).

Discussion

Quadruple immunosuppression regimens and sequential antilymphocyte antibody regimens have achieved good results in sensitized patients [14–17]. Nevertheless, conflicting results have been published [18–21], and to date we still lack formal proof of the advantage of prophylactic antilymphocyte antibodies for sensitized patients. Indeed, these regimens may also increase the risk of infections and neoplasia [19]. Moreover, since cyclosporin has been used, the effect of sensitization on acute rejection and graft survival may have been weakened [22–24]. At a time when promising new immunosuppressants are coming on to the market, it may be useful to assess the results we can achieve with our existing immunosuppressants and standard strategies.

The choice between anti-CD3 and antilymphocyte antibodies remains difficult. In a study carried out in sensitized patients, Vela compared ALG with OKT3 [12] and concluded that they were equally effective but that side effects were less frequent with ALG. Although ATG is more recent than ALG, it has been recognized as a potent immunosuppressive agent [25].

We found that induction therapy with ATG in sensitized kidney recipients gave a benefit when compared with triple therapy: the incidence of acute rejection episodes was reduced from 64 to 38%, 1-year graft survival increased from 76 to 89% and 1-year graft function was also improved. However, the causes of graft loss were not equally distributed, and non-immunological graft failures occurred mainly in the triple therapy group. When non-immunological graft failures were removed along with deaths, the difference between 1-year graft survivals was reduced from 13 to 7%. It might be argued that prophylaxis with ATG would not have avoided these graft losses in that group had they been given ATG. The severity of pathological damage did not appear to be modified by the induction regimen. The pathological features were graded retrospectively with the Banff classification, as the study was initiated prior to its publication. Unfortunately, sample sizes did not allow us to apply this classification to half of the biopsies. Nevertheless, we noticed the same percentage of rejections with vascular lesions which would have been grade II or III in the Banff classification. ATG induction also delayed the onset of the first rejection episode; in fact we encountered rejection crises occurring later than the third month post-transplantation. One should be aware of these late rejections which could jeopardize the results obtained with ATG.

Surprisingly, treatment with ATG did not induce many more early side effects or adverse reactions, but we must point out that rather low doses were used. There is a strong relationship between side effects and the duration of treatment [26]. Careful monitoring of T cells could also reduce the incidence of infections [27]. It is therefore logical to use a potent but carefully adjusted and short-lasting induction protocol.

The most striking result is that ATG induction was of benefit to all the sensitized patients and not only the highly sensitized patients. The highly sensitized patients exhibited a mild reduction in acute rejection episodes; they remain very difficult patients to treat. Usually, a lot of attention is paid to hypersensitized patients, whereas patients with lower levels of sensitization are often excluded from joint programmes and clinical studies. It has been reported that sensitized patients with a PRA $<$ 50% had a graft survival rate which compared favourably with those of more highly sensitized patients [3,28], but little is known about the clinical management of these patients. Our results support the systematic use of an induction therapy in all sensitized patients.

In conclusion, a prophylactic 10-day course of low-dose ATG, given to cyclosporin-treated sensitized kidney recipients, decreased the incidence of acute rejection episodes, increased graft survival and improved graft function. All sensitized patients, whatever their PRA level, benefited from this ATG induction therapy.

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Received for publication: 8.4.97
Accepted in revised form: 8.10.97