Transpl Int (1998) 11: 231–236

ORIGINAL ARTICLE

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A randomized prospective study comparing low-dose OKT3 to low-dose ATG for the treatment of acute steroid-resistant rejection episodes in kidney transplant recipients

Received: 27 June 1997
Received after revision: 15 October 1997
Accepted: 19 November 1997

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Abstract Acute steroid-resistant rejection episodes in kidney allograft recipients require treatment with antilymphocyte antibodies. Monoclonal anti-CD3 and polyclonal antilymphocyte antibodies have been widely used but seldom compared. Recent data have suggested that these antibodies could be used at reduced doses without jeopardizing their efficacy. In this study, we randomized renal transplant recipients who encountered a first acute steroid-resistant rejection episode to low-dose ATG or low-dose OKT3 treatment. Sixty patients were enrolled in the study. They received prophylactic immunosuppression with cyclosporin, azathioprine, and prednisolone. Treatment of biopsy-proven rejection consisted of a 10-day course of either ATG ($n = 31$) or OKT3 ($n = 29$). The total ATG dose was 484 ± 110 mg, i.e., 0.75 mg/kg per day. The total OKT3 dose was 32 ± 4 mg, i.e., 0.05 mg/kg per day. We compared reversion of rejection, side effects, immunodepression, and graft function. Reversion of rejection was similar in the two groups, although we noted a trend in favor of ATG. Results were 3% vs 10% early graft failures, 13% vs 23% overall graft failures, 28% vs 38% 3-month actuarial incidence of rebound rejection, and 89% vs 81% 1-year graft survival rate in the ATG and OKT3 groups, respectively. Tolerance was worse in the OKT3 group due to the first-dose syndrome. Infections and cancers occurred with the same frequency. ATG resulted in a deeper and longer decrease in peripheral lymphocyte subsets. Graft function was similar in the two groups. We conclude that low-dose ATG and low-dose OKT3 are equally effective in reversing steroid-resistant acute rejection. Tolerance was better with ATG, which also gave a more potent and longlasting immunodepression. The use of reduced doses of ATG and OKT3 did not appear to lessen their efficacy.

Key words Kidney transplantation, acute rejection, ATG, OKT3 · ATG, OKT3, rejection, kidney transplantation · OKT3, ATG, rejection, kidney transplantation · Rejection, OKT3, ATG, kidney transplantation

Introduction

In renal transplant recipients who experience acute steroid-resistant rejection episodes, treatment with antilymphocyte globulin (ALG) and later with antithymocyte globulin (ATG) and anti-CD3 monoclonal antibody (OKT3) has been widely reported [7, 13, 20]. Both ATG and OKT3, individually, are usually successful in reversing steroid-resistant rejection episodes, but overimmunosuppression leading to infections, cytomeg-

alovirus (CMV) diseases, and lymphomas [6, 9, 14] remains a common problem. Comparison of OKT3 with ATG has mainly been done in induction therapies; very few studies have compared them as treatment for acute rejection episodes.

OKT3 is usually given at a dose of 5 mg/day. This dose is known to be sufficient to block T-cell functions in in vitro assays [11]. However, this has not been confirmed by further clinical data. Recently, a number of studies have been designed to analyze the efficacy of reduced doses of OKT3. It has been shown that induction protocols with reduced doses of OKT3 are as efficient as those with normal doses in selected, nonsensitized patients [3, 16, 17] and in immunized recipients [8]. The only report on reduced doses in the treatment of established rejection was recently published [15] and concluded that a daily dose of 2.5 mg is just as effective as the standard 5-mg daily dose. Reduced doses of ATG have also been used in prophylactic protocols [1] and as rejection therapy [5]; not only were they effective, but they also induced fewer adverse reactions. Yet, more studies are needed to confirm these findings, and evaluations of low-dose OKT3 and low-dose ATG should be conducted together.

We performed a prospective, randomized study in kidney allograft recipients, comparing treatment of the first biopsy-proven, steroid-resistant acute rejection episode with either low-dose ATG or low-dose OKT3.

Materials and methods

Patient population

Included in the study were all kidney transplant recipients who, from 1992 to 1995, rejected their graft for the first time and did not respond to high-dose steroids. Sixty patients were enrolled, in accordance with all ethical standards. All patients received the same prophylactic immunosuppression, i.e., triple therapy consisting of cyclosporin (CyA), azathioprine, and prednisolone.

Study design

The clinical diagnosis of acute rejection was made in cases of delayed graft function or 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. Color duplex sonography, isotopic nephrography, whole blood trough levels, and peak CyA levels were used to eliminate any other cause of graft dysfunction. Any graft dysfunction that could not be explained eventually led to a biopsy. When acute rejection was clinically suspected, two boluses of 15 mg/kg methylprednisolone were administred every 2nd day. Steroid resistance was defined as the absence of a clear drop in serum creatinine after two steroid boluses. An 18-G or 16-G core biopsy specimen was then taken and immediately processed. Results were available the following day. The Banff classification was applied either retrospectively or prospectively. If acute rejection was confirmed on pathological examination and still no fall in creatinine was observed, patients were randomized either to the ATG group or to the OKT3 group. ATG, conditioned in 25-mg vials (Thymoglobulin, IMTIX Pasteur Mérieux Connaught), was given according to body weight: 25 mg per day for a body weight less than 40 kg, 50 mg/day for a body weight between 40 and 75 kg, and 75 mg/day for a body weight over 75 kg. OKT3 (Orthoclone, Cilag) therapy consisted of 5 mg daily for 3 days, followed by 2.5 mg daily for 7 days. The pharmacy split the 5-mg vials and prepared the half-dose syringes under sterile conditions. Another 5 mg/kg methylprednisolone bolus was given in both groups 1 h before the antibody to minimize the first-dose syndrome. CyA and azathioprine were not withdrawn during antibody treatment.

Laboratory monitoring

Peripheral CD2- and CD3-positive cells were monitored to ensure the efficacy of the reduced doses. A blood sample was taken prior to treatment and every 2nd day. Mononuclear cells, obtained by Ficoll-Hypaque centrifugation, were stained with fluoroscein-labelled anti-CD2 and anti-CD3 monoclonal antibodies. The $CD2^+$ and $CD3^+$ T-cell count was estimated by immunofluorescence microscopy. The intention of treatment was to maintain the T-cell count at 0%.

The absolute number of total lymphocytes and of CD3, CD4, and CD8 lymphocytes was counted on flow cytometry before, at the end of, and 45 days after the antibody treatment.

Study end points

The primary criterion of efficacy was reversion of the rejection episode. We took into account early (within 3 months) and overall (at last follow-up) graft losses, the occurrence of further acute rejection episodes, and graft survival rates according to Kaplan-Meier. We also considered as secondary end points side effects, immunodepression, and graft function. Fever, hematological and metabolic disorders, and all other adverse effects were taken into account within 3 weeks of the onset of the treatment. Infectious complications were recorded within 3 months, cancers and deaths without any limitation. CMV asymptomatic serological conversions, as well as clinical CMV diseases, were considered as CMV infections.

Results

Demography and treatment

Thirty-one patients were treated with ATG and 29 with OKT3. The ATG and OKT3 groups were similar with regard to pretherapeutic conditions including duration of dialysis, recipient age, maximum PRA, HLA-A/B/DR mismatch, and second grafts (Table 1). We only noticed a shorter cold ischemia time in the ATG group than in the OKT3 group (27 ± 8 vs 32 ± 9 h, $P = 0.03$). The mean time of rejection onset was 46 days in the ATG group (median 12 days) and 43 days in the OKT3 group (median 20 days; P = NS). The gradation of pathological damage was identical with 38% vs 29% minimal lesions, 22% vs 38% grade I, 22% vs 24% grade II, and 17% vs 10% grade III in the ATG group and the OKT3 group,

Table 1 Patient demographics

	ATG	OKT3	*P*
Number	31	29	
Second transplantation	3 (10%)	1 (3%)	NS
Duration of dialysis (months)	47 ± 68	32 ± 42	NS
Females	7 (24%)	7 (22%)	NS
Recipient age	43 ± 13	44 ± 13	NS
Donor age	39 ± 10	40 ± 17	NS
Maximum PRA	20 ± 30	14 ± 27	NS
PRA > 80%	3 (10%)	3 (10%)	NS
HLA-A/B/DR mismatch	3.1 ± 0.7	2.6 ± 0.9	NS
Warm ischemia (min)	21 ± 7	23 ± 13	NS
Cold ischemia (h)	27 ± 8	32 ± 9	0.03

respectively (*P* = NS). Before steroids were given, serum creatinine was 477 ± 334 μmol/l in the ATG group and 447 ± 327 μmol/l in the OKT3 group (*P* = NS). After steroids but before antibody treatment, serum creatinine was 513 ± 348 μmol/l in the ATG group and 448 ± 289 μmol/l in the OKT3 group (*P* = NS).

Low-dose ATG resulted in a cumulative 484 ± 110 mg treatment, corresponding to an average of 0.75 mg/kg per day. Similarly, low-dose OKT3 resulted in a cumulative 32 ± 4 mg treatment, corresponding to an average of 0.05 mg/kg per day. No circulating $CD2^+$ or $CD3^+$ cells were present in any of the patients within 2 days after the first injection or throughout the treatment.

Reversal of rejection

Three months after treatment, one graft (3%) was lost in the ATG group and three (10%) in the OKT3 group (*P* = NS). At last follow-up (531 ± 331 days), four (13%) and six grafts (21%) were lost respectively (*P* = NS). In the ATG group, graft failures were due to rejection in one patient and to death in three patients. In the OKT3 group, graft failures were due to rejection in four patients, to surgical complication in one patient, and to death in one patient. Hence, immunological failures amounted to 3% in the ATG group and to 13% in the OKT3 group. The actuarial incidence of rebound rejection episodes is given in Fig. 1. Three months after the end of treatment, the cumulative rate of re-rejection was 28% in the ATG group and 38% in the OKT3 group (*P* = NS). The actuarial graft survival was 96% vs 90% at 3 months, 93% vs 90% at 6 months, and 89% vs 81% at 12 months in the ATG group and the OKT3 group, respectively (Fig. 2, *P* = NS).

Tolerance

Fever was more frequent in the OKT3 group than in the ATG group (52% vs 6%, *P* = 0.001), mainly due to the first-dose syndrome with chills, headache, myalgia, and tremor. Three serum sicknesses occurred in the ATG group; one inferior limb arterial thrombosis and one deep venous thrombosis occurred in the OKT3 group. We noted 22% vs 17% leukothrombopenia, 39% vs 45% CMV infections, 10% vs 7% herpes virus infections, 6% vs 10% severe bacterial infections, 4% vs 7% urinary tract infections, 6% vs 10% monoclonal gammopathies, and 0% vs 7% solid tumors in the ATG and the OKT3 groups, respectively. Three patients died in the ATG group on days 9, 290, and 369 post-transplantation, respectively. One patient died in the OKT3 group on day 36.

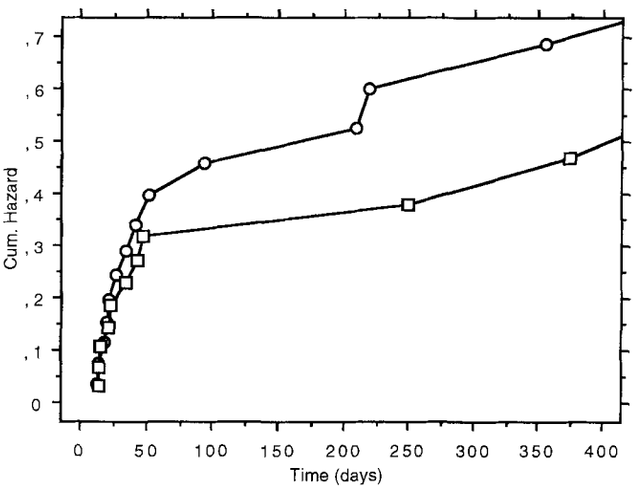

Fig. 1 Kaplan-Meier cumulative hazard plot for rebound rejection after OKT3 (-○-) and ATG (-□-) treatment for a first acute rejection episode

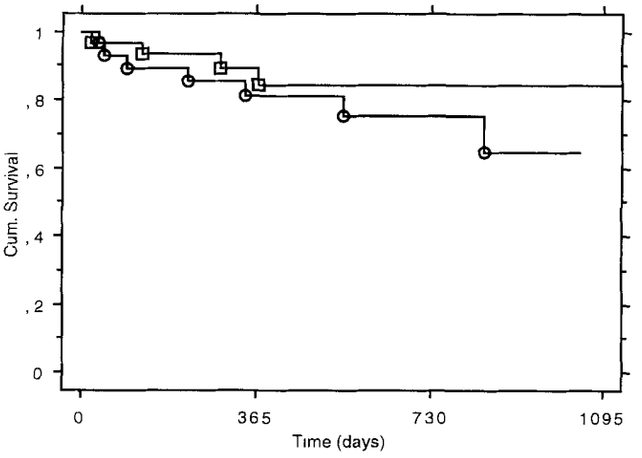

Fig. 2 Graft survival curves in patients treated with OKT3 (-○-) and ATG (-□-) for a first acute rejection episode

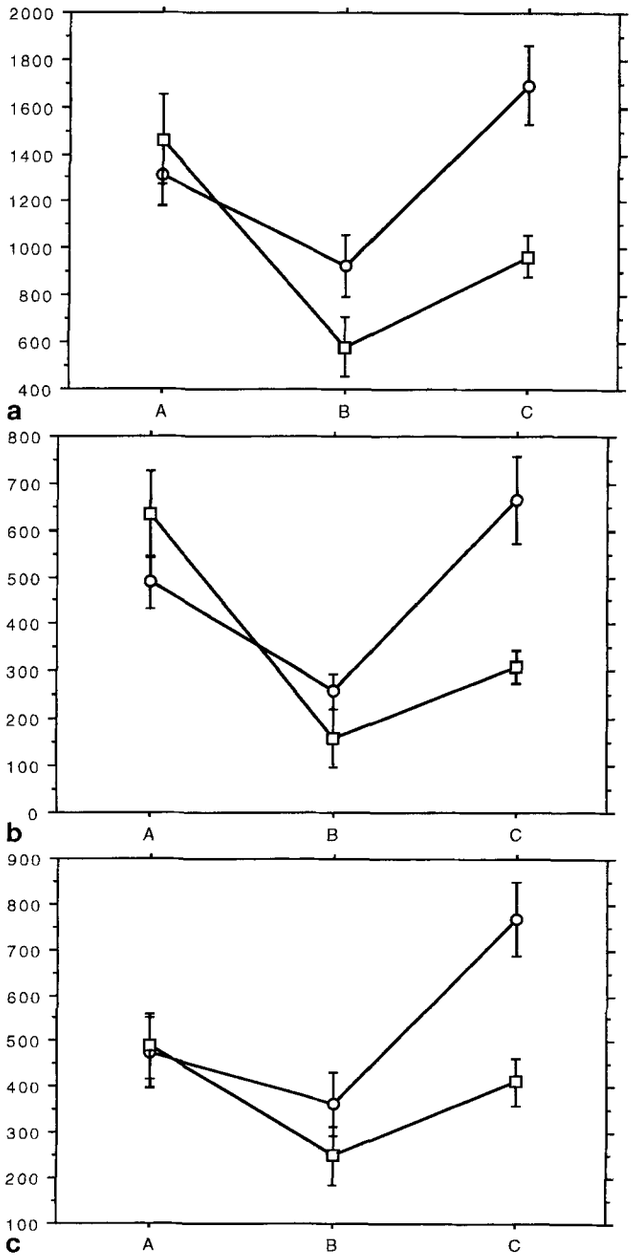

Fig. 3a–c Absolute count of: **a** peripheral total lymphocytes **b**, CD4 lymphocytes, and **c** CD8 lymphocytes in OKT3 group (–○–) and in ATG group (–□–). Values are mean cells per mm^3 ± SE measured **A** before, **B** at the end of, and **C** 45 days after antibody treatment. P = NS for A and B; $P < 0.001$ for C

Immunodepression

Before treatment, the absolute number of lymphocytes and of CD3, CD4, and CD8 lymphocytes was similar in both groups. At the end of treatment, all of these lymphocyte subsets were lower in the ATG group, but the difference was not statistically significant. Forty-five days after treatment, there were significantly fewer lymphocytes and CD3, CD4, and CD8 cells in the ATG group than in the OKT3 group (Fig. 3).

Graft function

On the basis of serum creatinine, graft function was similar in both groups at the end of treatment (306 ± 253 vs 237 ± 166 μmol/l) and 45 days after treatment (245 ± 181 vs 242 ± 134 μmol/l).

Discussion

Presently, there is a tendency to reduce the dose of antilymphocyte antibodies given to kidney recipients. Published data provide evidence that doses below the manufacturers' recommendations do not hamper their efficacy. The use of reduced doses first appeared to be effective in prophylactic regimens [1, 3, 16, 17). Then, low-dose OKT3 [15] and low-dose ATG [5] were found to be as effective as standard doses in reversing acute rejection episodes. The effect of the duration of antilymphocyte therapy on the infection rate has clearly been demonstrated [21]. The optimal dose at which these antibodies should be given is still subject to debate.

In the present study, the low doses of antibodies given resulted in satisfactory treatment of acute rejection episodes: within 3 months after treatment, 93% of the grafts were functioning, and the overall 1-year graft survival rate was 85%. It was not the purpose of this study to compare and contrast efficacy and side effects between full-dose and low-dose regimens. However, we did previously conduct a very similar study in our center comparing full doses of OKT3 and ATG [2]. The reversion rate of acute steroid-resistant rejection episodes was 90% and the 1-year actuarial graft survival rate was 80%. Even though it is not possible to use historical data to make statistical comparisons, reversion of rejection and graft survival were not, in our experience, reduced by the use of low doses.

Since the immunosuppressive power of antilymphocyte antibodies depends on their effects on T lymphocytes, T-cell monitoring has been proposed for a better adjustment of the doses [10]. In this study, a full 5-mg dose of OKT3 was given for 3 days and the subsequent daily doses were reduced to 2.5 mg. In a pre-study phase, we tried to give the 2.5-mg dose from the 1st day onwards, but it took longer to eliminate the circulating $CD3^+$ cells. Hence, to avoid insufficient immunosuppression, we chose the present study design. A similar two-step procedure has already been published, but in uncontrolled, nonrandomized studies [8, 19]. ATG was

given according to body weight. The mean dose that the patients actually received was 0.75 mg/kg per day, which represents 60% of the recommended dose. Adjusting ATG doses to the CD3 count, Abouna et al. obtained a similar result, reducing ATG to 64% of the recommended full dose [1]. Given the CD2 and CD3 peripheral T-cell count in all of our patients, we were clearly able to avoid insufficient immunosuppression.

Numerous studies have compared ATG to OKT3 in induction protocols, but few studies are available that enable one to evaluate them as treatment for acute kidney rejection episodes [12]. To the best of our knowledge, there is no evidence to support the superiority of either, although it has been claimed that OKT3 is more efficient in treating acute vascular rejection [18]. As for the reversion of rejection, the present study did not show a statistical difference between results obtained with OKT3 and those obtained with ATG. Nevertheless, we noticed a trend in favor of ATG treatment. Firstly, prednisolone given prior to OKT3 did not prevent first-dose syndrome, whereas early tolerance of ATG was better. Secondly, there were fewer graft losses, fewer immunological failures, fewer rebound rejections, and a better graft survival rate in patients treated with ATG. Lastly, ATG treatment induced a longlasting diminution in circulating lymphocytes. Given the fact that ATG was also found to be more effective and to have fewer side effects than OKT3 in an earlier prophylactic trial [4], we suggest that ATG might be a more potent immunosuppressant than OKT3.

We conclude that both ATG and OKT3 can be used to treat acute steroid-resistant rejection episodes in kidney recipients. The use of low doses does not jeopardize the efficacy of these antibodies and can be proposed as a safe and efficient procedure.

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