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# Effect of ATG prophylaxis in sensitized and non-sensitized kidney graft recipients

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**Abstract.** In our effort to find an optimum immunosuppressive protocol for kidney transplantation we introduced two forms of ATG prophylaxis: 1. high-dose single-bolus prophylaxis (9 mg/kg) in non-sensitized patients (PRA < 5%); and 2. low-dose 8-day prophylaxis (1.5–3.0 mg/kg) in sensitized patients (PRA > 5%). A total of 204 kidney graft recipients were included in this study and treated with a triple-drug therapy (TDT). In comparison with TDT-treated controls, in sensitized patients the 8-day ATG prophylaxis resulted in a reduced rate of rejection episodes (25.5% vs 47%), an improved 1-year graft survival (82% vs 71%) and patient survival (94% vs 90%). In non-sensitized patients the high-dose single-bolus ATG prophylaxis induced a T-cell lymphopenia lasting 4 to 5 days and, in comparison with the corresponding controls, resulted in a shortened hospital stay (31.2 days vs 36.7 days), a reduced rate of rejection episodes (25.5% vs 53%), an improved 1-year graft survival (92% vs 86%) and patient survival (100% vs 94%).

**Key words:** Kidney transplantation – ATG prophylaxis – Rejection episodes – Graft survival – Patient survival

In 1990 Kormos et al. [14] showed that prophylactic rabbit anti-human thymocyte globulin (RATG) achieves a major reduction in early cardiac graft rejection without an increased risk of major opportunistic or bacterial infections, and concluded that RATG would be an important adjunct to contemporary immunosuppressive protocols. Grino et al. [6], using horse ALG, also reported a low incidence of acute rejection episodes after cadaveric renal transplantation. Encouraged by these results we introduced two forms of ATG prophylaxis depending on the sensitization status of the prospective recipient. Sensitized recipients [panel reactive lymphocytotoxic antibodies (PRA) > 5%] received eight consecutive ATG infusions starting intraoperatively in addition to triple-drug therapy (TDT) consisting of azathioprine (AZA), cyclosporine (CyA) and low-dose prednisolone (PRED). In non-sensitized patients (PRA < 5%) we inaugurated a new treatment protocol consisting of an intraoperative high-dose single-ATG bolus in addition to TDT.

These treatment protocols were chosen because in our hands the graft survival in sensitized recipients was inferior to that in non-sensitized recipients [3]. The rationale of the protocols was to produce maximal immunosuppression when the recipient was most likely to respond to the new organ.

## Materials and methods

### *Study population*

A total of 204 patients who received their cadaveric renal transplant at the Kidney Transplant Centre Berlin-Friedrichshain from April 1987 to November 1990 were included in this study.

### *Immunosuppressive protocol*

All patients received AZA (4 mg/kg) in their dialysis unit immediately before being called to the transplant centre. Methylprednisolone (500 mg intravenously) was given during transplantation, and postoperatively the patients received 40 mg for 7 days, subsequently switching to 35 mg/day PRED for 14 days. A maintenance dose of 10–15 mg/day PRED was then continued for 12 months. Oral CyA was started within 24 h of surgery. The patients received 6 mg/kg divided in two daily doses. During the first postoperative week a maintenance CyA level of 100 ng/ml (RIA, ICN-STAR, SORIN), and thereafter of 200 ng/ml, was the aim. AZA was restarted after surgery at an oral dose of 1 mg/kg and maintained as long as the leucocyte count was greater than 4000/mm$^3$.

### *Monitoring for rejection and infection*

For the diagnosis of rejection the following clinical and laboratory signs were decisive: enlargement and tenderness of the graft, increase in serum creatinine and C-reactive protein, concomitant

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change in blood urea nitrogen, oliguria, immunoglobulinuria, sonographic changes, immunoactivation in fine-needle aspiration cytology [6], biopsy-proven rejection. Rejection was treated with PRED, 5 mg/kg for 5 days, or with ATG, 1.5–3 mg/kg for 8–10 days depending on the T-cell count ($< 200/mm^3$). Infections were classified as either major or minor. Major infections included pneumonia, pyelonephritis, cytomegalovirus (CMV) disease and invasive fungal infection.

CMV infection was diagnosed by the detection of CMV-specific IgM antibodies, a fourfold or greater increase in CMV-specific IgG antibodies (CMV-ELISA, Enzygnost Behring, Marburg, FRG) and/or the fluorescence microscopic detection of CMV antigen-carrying peripheral blood cells or fine-needle-aspirated kidney graft cells [7] by means of monoclonal antibodies (Clonab-CMV, Behring, Marburg, FRG). CMV disease was diagnosed using clinical criteria including leucopenia, spike-like fever, elevation of aminotransferases, thrombocytopenia, deterioration of graft function etc. The treatment of CMV disease depended on the severity of clinical symptoms and included the application of human immunoglobulins with a high content of CMV-specific antibodies (CYTOTECT, Biotest, Dreieich, FRG), the reduction or cessation of AZA for several days and sometimes the prophylactic application of antibiotics.

### *ATG prophylaxis*

We used rabbit anti-human T-lymphocyte globulin (ATG) (Fresenius, Oberursel, FRG) in two different protocols. Non-sensitized patients (PRA < 5%) received 9 mg/kg ATG intravenously just before the anastomoses were completed. Sensitized patients (PRA > 5%) and patients waiting for a second graft were given 1.5–3.0 mg/kg ATG intravenously for 8 days beginning with the first infusion also intraoperatively before the anastomoses were completed. The daily ATG doses depended on the T-cell count, which needed to be lower than $200/mm^3$.

### *T-cell count*

In order to monitor the ATG effect the cell count was determined three times a week using the spontaneous rosette formation test [11] according to the previously published method [12]. More recently we have been able to confirm and extend our results by FACS analyses (data not published).

## Results

### *Sensitized recipients*

In this group, 51 out of 102 sensitized (PRA > 5%) or re-grafted recipients received an 8-day ATG prophylaxis. The basic immunosuppression in both groups consisted of AZA, PRED and CyA. The results are summarized in Table 1. There was no difference in the postoperative hospital stay (ATG vs non-ATG: $38.9 \pm 25.3$ days vs $39.6 \pm 25.3$ days). However, the frequency of rejection crises up to discharge was significantly lower in the prophylaxis group (25.5% vs 47%), and the proportion of functioning grafts was increased by 10%. The improved graft survival was still evident 1 year after transplantation. The 1-year patient survival was also slightly better in the prophylaxis group (94.1% vs 90.2%). In the ATG group three patients did not survive, the causes of death being sepsis (one) and heart failure (two). In the non-ATG group five patients did not survive, the causes of death being sepsis (one), embolism (one), myocardial infarction (one) and heart failure (two). With respect to CMV infection the proportion of secondary infections was significantly higher in the ATG prophylaxis group, and also in this group the number of patients who experienced oligosymptomatic CMV disease (leucocytopenia $< 4000/mm^3$ and fever $\geq 38°$ for at least 2 days) was higher (25/51 vs 14/51). This was also reflected by a higher consumption of Cytotect (Biotest, FRG) in the prophylaxis group (total 4080 ml vs 2760 ml). No difference was seen in the occurrence of polysymptomatic severe CMV disease ($n = 3$ for each group).

### *Non-sensitized patients*

In this group, 51 out of 102 non-sensitized TDT-treated kidney graft recipients received intraoperatively one infusion of 9 mg ATG/kg. No serious side effect was observed. At 24 h post-transplant, the absolute number of T cells (RFC) was decreased from $916 \pm 390/mm^3$ to $187 \pm 121/mm^3$ (Fig. 1). This T lymphopenia lasted until the fourth post-transplant day ($284 \pm 173/mm^3$). Thereafter, a slow increase was recorded, reaching the T-cell level of the TDT recipients by day 7. Besides this T lymphopenia, the high-dose single-bolus ATG prophylaxis, in comparison with the controls (Table 2), resulted in a shortened post-transplant hospital stay (31.2 vs 36.7 days), and a reduced rate of rejection episodes (25.5% vs 53%). This is also reflected by the consumption of drugs needed for treatment of rejections (ATG vs non-ATG group: PRED, 19.52 vs 21.96 g; ATG, 2.8 vs 18.8 g; OKT3, 0 vs 45 mg).

With regard to CMV infection diseases there were no differences between the groups, and life-threatening epi-

**Table 1.** Sensitized kidney graft recipients: triple-drug therapy

| | Without 8-day ATG prophylaxis | With 8-day ATG prophylaxis |
|---|---|---|
| Patients ($n$) | 51 | 51 |
| Follow-up time | 4.87–1.90 | 9.89–10.90 |
| Hospital stay (days, mean) | 39.6 | 38.9 |
| Number of recipients with rejection crises | 24 (47%) | 13 (25.5%) |
| CMV infections | | |
| primary ($n$) | 5/7 | 5/7 |
| secondary ($n$) | 17/44 | 30/44 |
| CMV diseases | | |
| light ($n$) | 19/22 | 32/35 |
| severe ($n$) | 3/22 | 3/35 |
| Patient survival (%) | | |
| 3 month | 94 | 94 |
| 6 month | 92 | 94 |
| 12 month | 90 | 94 |
| Transplant survival (%) | | |
| 3 month | 78 | 82 |
| 6 month | 74 | 82 |
| 12 month | 71 | 82 |

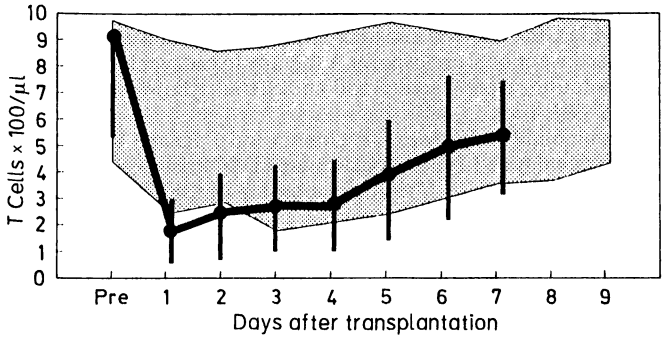


**Fig. 1.** Absolute number of sheep erythrocyte rosette-forming cells (E-RFC) during in the first week after kidney transplantation. The *black line* represents the mean ± 1 standard deviation of 51 recipients intraoperatively treated with 9 mg/kg ATG-Fresenius in addition to the triple-drug therapy. The *dotted area* represents the absolute number of RFC (mean ± 1 s.d.) in 51 only triple-drug treated recipients.

sodes were not observed. The amount of CMV gammaglobulin infused was almost comparable in the ATG and in the non-ATG groups (7050 vs 6530 ml). With respect to the long-term results, both the 1-year graft survival (92% vs 86%) and patient survival (100% vs 94%) were clearly improved. It should be noted that three out of four recipients in the prophylaxis group who were on dialysis after 1 year had a primary non-functioning graft. In the non-prophylaxis group, three patients did not survive, the causes of death being sepsis (one), lung embolism (one) and cerebral bleeding (one).

## Discussion

In an effort to find an optimum immunosuppressive protocol for organ transplantation many centres are using polyclonal [4–6, 8, 10, 14, 16–18], or monoclonal [1, 2, 9, 15, 16, 19, 20] anti-T-lymphocyte antibodies as prophylaxis in addition to conventional immunosuppressive drugs. The protocols, as well as the antibodies used, are very different. The rationale for using these agents is, first, to reduce the amount of CyA in the early post-transplant period in order to avoid nephrotoxicity, second, to produce maximal immunosuppression when the host is most likely to respond to the new organ, and, third, to reduce the probability of rejections without increasing the risk of infections.

Our data confirm the previously reported excellent overall results with the simultaneous use of ALG, CyA and steroids [4, 6], ALG, AZA and steroids [8, 18] or ALG, AZA, CyA and steroids [10] as induction therapy after kidney transplantation. In contrast to Grundmann et al. [8] and Fries et al. [4], who gave ALG for 14 days and reported a high rate of intolerance and unexplainable fever [8] or an increased rate of clinical CMV infections [4], our protocol included only eight ATG infusions starting intraoperatively.

To demonstrate a beneficial effect of our quadruple-drug induction therapy also in high risk patients, we treated 51 presensitized or regrafted patients according to this protocol. In comparison with TDT-treated sensitized kidney graft recipients (control group) the quadruple-drug induction therapy improved the 1-year graft survival from 71% to 82% and the 1-year patient survival from 90% to 94%, and reduced the number of patients who experienced rejection crises from 47% to 25.5%. This is in contrast to the results of Illner et al. [10] who reported a 55% frequency of rejection episodes in a comparable group. Thomas et al. [18] reported a shorter hospital stay and a cadaver kidney graft survival rate of 92% in 40 (but only 38% presensitized) quadruple-drug-treated recipients. The ATG was also given according to a protocol to reduce the level of total circulating T cells to below 200/mm$^3$.

Thus, the prophylactic use of ATG in addition to the conventional TDT improves the graft survival also in presensitized recipients, seems to be safe because early nephrotoxic side effects were not observed (trough level 100 ng/ml during the first postoperative week) and severe infectious complications did not increase.

In our second series, a newer technique of immunosuppression, high-dose single-bolus ATG and TDT, was used in 51 non-sensitized recipients. The overall patient and graft survival after 12 months (100% and 94%, respectively) was excellent. Graft losses occurred in four patients in the study group, but only one due to rejection. Three out of these four grafts were primarily non-functional. However, this situation could still be improved by a better graft survival rate.

Bearing in mind particularly the cost of transplantation, the induction therapy reduced the rate of rejection episodes from 53% to 25.5%, shortened the hospital stay on average from 36.7 days to 31.2 days and did not in-

**Table 2.** Non-sensitized kidney graft recipients: triple-drug therapy

| | Without ATG bolus | With ATG bolus |
|---|---|---|
| Patients (*n*) | 51 | 51 |
| Follow-up time | 3.89–2.90 | 2.90–11.90 |
| Hospital stay (days, mean) | 36.7 | 31.2 |
| Number of recipients with rejection crises | 27 (53%) | 13 (25.5%) |
| CMV infections | | |
| primary (*n*) | 19/22 | 17/20 |
| secondary (*n*) | 21/29 | 17/20 |
| CMV diseases | | |
| light | 39/40 | 32/34 |
| severe | 1/40 | 2/34 |
| Patient survival (%) | | |
| 3 month | 96 | 100 |
| 6 month | 94 | 100 |
| 12 month | 94 | 100[a] |
| Transplant survival (%) | | |
| 3 month | 92 | 94 |
| 6 month | 88 | 94 |
| 12 month | 86 | 92[a] |

[a] 8/51 recipients at time of evaluation only 10 month post-transplantation with very well functioning graft

fluence the rate of infectious complications. It should be noted that ATG prophylaxis did reduce the actual rejection rate as opposed to only delaying the onset of rejections. During the first post-transplant week, the CyA trough values were only 100 ng/ml in order to reduce early nephrotoxic side effects. An optimal intraoperative and postoperative immunosuppression was induced by the high-dose single-ATG bolus leading to a T lymphopenia lasting at least 4 days. During this time the first, and may be decisive, contact between host and graft takes place.

Thus, the excellent results of high-dose single-bolus ATG prophylaxis in non-sensitized kidney graft recipients encouraged us to extend this protocol also to sensitized recipients.

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