IN VITRO IMMUNORESPONSIVENESS IN RECEPIENTS OF CAUDAERIC RENAL ALLOGRAFTS DURING ATG THERAPY

H Grosse-Wilde, H D Jakubowski, F W Eigler, E K Kuwert
University Hospital of Essen, Essen, FRG

Summary
Anti-human-T-cell-globulin (ATG-Fresenius) was given prophylactically in a fixed dose of 2mg per kg bodyweight to 32 renal allograft recipients in addition to a conventional immunosuppressive regimen, over a period of 20 days. ATG therapy resulted in a significant decrease of circulating T-lymphocytes, whereas B-lymphocytes remained unaffected. The mitogen reactivity during therapy paralleled the T-lymphocyte profile with a maximum decrease on day 8. With in vitro testing of ATG immunoresponsiveness it could be demonstrated that patients with rejection episodes after transplantation reacted differently from patients without signs of rejection. Actuarial patient and graft survival was about 5% higher in the ATG treated group of patients than in retrospective controls.

Introduction
In addition to the conventional immunosuppressive therapy after renal transplantation (Cortisone, Azathioprine) the use of specific antisera directed preferably towards human T-lymphocytes appears to be a valuable concept. An anti-human-T-cell-globulin (ATG-Fresenius) produced in rabbits immunised with a T-lymphoblast cell line (Jurkat) was therefore given to 32 cadaveric kidney allograft recipients. Before and during ATG administration the immunocompetence of these patients was followed up by T- and B-cell enumeration and in vitro lymphocyte transformation with four different mitogens: Phytohaemagglutinin (PHA), Concanavalin A (Con A), Pokeweed Mitogen (PWM), and ATG-Fresenius, which was found to be mitogenic in previous tests.

Materials and methods
The patient group (age 3–58 years) comprised 32 renal allograft recipients who received prophylactically 2mg ATG-Fresenius per kg bodyweight intravenously
from the first postoperative day over a period of 20 days. In addition a normal immunosuppressive regimen was administered consisting of Prednisone (30mg/d) and Azathioprine. The Azathioprine dose was adjusted to the peripheral white blood count.

On days 4, 8, 12, 16, 20 during ATG therapy 20ml heparinised blood (50U/ml) were separated on a Ficoll gradient to isolate the peripheral blood lymphocytes [1]. T-cell enumeration was performed by rosetting the lymphocytes with sheep red blood cells [2]. Lymphocytes rosetting with more than 3 sheep red blood cells were counted as positive. B-cell enumeration was carried out by immunofluorescence with FITC labelled rabbit-anti-human-Ig. The in vitro stimulation assays were done by a micromethod using $5 \times 10^4$ lymphocytes per well in quadruplicates. The following mitogens were used in 2 different concentrations: PHA (20, 40µg/ml), ConA (250, 500µg/ml), PWM (20, 40µg/ml), and ATG-Fresenius in a protein concentration of 2 and 4mg/ml respectively. All the concentrations used elicited the highest $^3$H-Thymidine uptakes in healthy controls.

Since the values of $^3$H-Thymidine uptake rates in the patient group were highly correlated ($r > 0.85$) the mean values of both mitogen concentrations are given in the figures.

Results

With regard to the T-/B-cell profile during ATG therapy, there was a clear drop of circulating T-lymphocytes, most prominent at day 8 and at that time statistically significantly different ($p < 0.05$) compared to the values before therapy (Figure 1). The B-cell counts remained stable during therapy with a slight but not

**Figure 1.** T-/B-cell profile (± SEM) in 32 renal allograft recipients before and during prophylactic ATG-therapy. *Statistically significant difference ($p < 0.05$) compared to day 0
significant increase (maximum day 16).

All the mitogens used revealed a clear drop of in vitro immunoreactivity on day 8 followed by a steady increase of stimulation values at the end of therapy, whereas for ATG an even higher stimulation level was reached (Figure 2). Looking at the individual response profiles for each patient and each mitogen it became apparent that among the ATG responses only, two different groups exist which could be related to rejection episodes observed (Figure 3): Patients without rejection (n = 21) exhibited on days 4 and 8 a marked reduction of ATG in vitro responsiveness whereas patients with rejection episodes (n = 11) did not demonstrate such a reduced response on these days. In both groups, however, the ATG in vitro response increased during the last 10 days of ATG therapy.

The actuarial graft survival of the ATG treated kidney recipients 12 months post transplantation was 71%, which is about 10% higher than in the retrospective controls (Figure 4). Furthermore patient survival in the ATG group was more than 5% higher than in the controls.

**Figure 2.** In vitro reactivity towards 4 different mitogens of 32 renal allograft recipients before and during prophylactic ATG-therapy
Figure 3. Different effect of ATG-therapy in renal allograft recipients with (n = 11) and without (n = 21) rejection episodes with regard to in vitro response towards ATG. ATG in vitro response on day 0 was taken as 100%. * Significantly different (p < 0.05)

Figure 4. Actuarial survival of patients and renal allografts treated prophylactically with ATG (n = 32) compared with retrospective controls (n = 100)
Conclusions

ATG-Fresenius was given prophylactically (fixed dose 2mg/kg bodyweight) to 32 kidney graft recipients in addition to a normal immunosuppressive regimen over the period of 20 days post transplantation. Compared to values obtained before treatment there was a statistically significant decrease of circulating T-lymphocytes on day 8, whereas the number of B-lymphocytes remained unaffected indicating that the ATG preparation used acts preferably on T-lymphocytes. The mitogen responses paralleled the T-cell profile. However during the last 10 days of ATG therapy the stimulation values reached almost those found before therapy, which could reflect a reactive liberation of T-lymphocytes from central pools. In vitro stimulation with ATG-Fresenius seems to discriminate between patients having rejection episodes and those who do not, since in the rejection group ATG in vitro responses remained high during the first week of therapy, whereas patients with no rejection had an immediate reduction of ATG in vitro responsiveness.

Besides the in vitro parameters ATG-Fresenius administration has in our hands a favourable and steroid sparing effect on kidney graft and patient survival. It remains to be studied as to whether the fixed ATG dose of 2mg per kg bodyweight can or should be increased in patients at risk for rejection.

References