PRETRANSPLANT T CELL LEVELS AND RENAL ALLOGRAFT SURVIVAL

C H Uittenbogaart, B J Robinson, M H Malekzadeh, A J Pennisi, R B Ettenger, R N Fine

University of Southern California School of Medicine and Dialysis and Transplant Program, Childrens Hospital of Los Angeles, Los Angeles, California, USA

Summary

Total rosette forming cell (TRFC) levels were measured in 50 paediatric patients awaiting cadaveric renal transplantation.

Preliminary data show a statistically significant difference in allograft survival in patients with low TRFC levels pretransplant as compared with patients with medium or high TRFC levels. Pretransplant TRFC levels may be predictive of a non-responder status and portend a favourable renal allograft outcome.

Introduction

A significant factor affecting renal allograft survival in man is the immunological responsiveness of each potential recipient. Attempts have been made to detect the degree of responsiveness prior to transplantation in order to predict allograft outcome.

Thomas et al [1,2] and Jones et al [3] reported that pretransplant T cell functions as determined by the degree of mitogenesis was an indicator of graft prognosis; however, Kerman et al [4,5] found no prognostic value in lymphocyte response to PHA in a relatively small group of recipients.

During the past 2 years at Childrens Hospital of Los Angeles (CHLA) serial peripheral blood T cell levels as total rosette forming cells (TRFC) were determined in all patients awaiting transplantation.

This preliminary report presents data on the relationships between pretransplant TRFC levels and cadaveric renal allograft survival.

Materials

TRFC levels were determined pretransplant in 50 patients aged 2–19 years, who received a cadaveric renal allograft at CHLA. Antithymocyte globulin (ATG) (Upjohn Company, Kalamazoo, Michigan) was added to the regular immuno-
suppressive regimen in 28 patients and 22 control patients received only prednisone and azathioprine in the dosage schedules previously described [6]. All patients were at risk for more than 3 months post-transplant at the time of this report.

Methods

Baseline TRFC levels were determined on at least 3 separate occasions prior to transplantation while the patients were undergoing dialysis. The mean pretransplant TRFC level was calculated for each patient. The distribution curve of the mean TRFC's was divided into equal tertiles and these were designated as low, medium and high TRFC groups. The low TRFC group had TRFC levels < 1101/mm³, the medium TRFC group had levels of 1101–1343 TRFC/mm³ and the high TRFC group had levels > 1343 TRFC/mm³. The number of recipients in the low, medium and high TRFC groups were 17, 16 and 17 respectively. Allograft survival was computed by actuarial methods; Mantel-Cox [7] and Breslow [8] statistical analyses were used to evaluate the data.

Rosette Assay

10ml heparinised blood was obtained from the patient and from a normal individual. The blood was layered over ficoll hypaque to separate the lymphocytes. Adjusted lymphocytes (5 x 10⁶/ml) were incubated in a 37° water bath for 40 minutes. 0.05ml of adjusted sheep red blood cells (SRBC) (1.25 x 10⁸/ml) were added to 0.05ml of adjusted lymphocytes, centrifuged and placed in an ice bath for 120 minutes to form rosettes. Rosette forming cells (RFC's) and non RFC's were counted using a haemacytometer. Any lymphocyte with 4 or more adherent SRBC's was considered a rosette. All tests were performed in triplicate. White blood cell and differential counts were determined in the patient and in the normal individual. RFC's were expressed as percent RFC's of total lymphocyte count and as TRFC's/mm³.

Results

The TRFC levels of the pretransplant patients (1243 ± 477) were significantly different from those of the normal individuals (1591 ± 334) (p < 0.001).

Actuarial allograft survival rates were statistically significantly different in the low TRFC group when compared with the groups with medium and high TRFC levels (p < 0.05) (Mantel-Cox test). The 6, 12 and 18th month allograft survival rates are 94 ± 5.7%, 94 ± 57% and 94 ± 5.7% for the low TRFC group, 67 ± 12.2%, 50 ± 13.7% and 41 ± 13.9% for the medium TRFC group and 64 ± 11.9%, 64 ± 11.9% and 53 ± 13.9% for the high TRFC group respectively (Figure 1). There was no statistically significant difference when comparing allograft survival rates of ATG treated and control patients in relationship to the pretransplant TRFC levels (Figure 2).
Figure 1. Actuarial graft survival of low, medium and high TRFC groups. Low vs. medium and high TRFC group p < 0.05

Figure 2. Actuarial graft survival of ATG treated and control patients divided into low, medium and high TRFC groups
Discussion

It would be advantageous to be able to segregate potential allograft recipients into responder and non-responder groups by means of in vitro tests. Such categorisation would be valuable from the standpoint of patient counselling and would also facilitate individualisation of post-transplant management. In addition, it would aid in designing protocols to test the efficacy of various immunosuppressive regimens. Various attempts at such categorisation have been reported.

In 1972, Opelz et al [9] categorised recipients into responder and non-responder groups on the basis of humoral presensitisation. Those patients who failed to develop lymphocytotoxic antibodies following a challenge of repeated blood transfusions during a year of dialysis were designated as non-responders. Renal allograft survival was significantly better in the non-responders than in the group of recipients who developed lymphocytotoxic antibodies (responders). Subsequent reports have shown reduced allograft survival rates in highly presensitised recipients [10,11]; however, the data have not been uniform [12,13,14] and the predictive value of presensitisation remains equivocal.

Rolley et al [15] attempted to detect the immunological responsiveness of transplant candidates by monitoring their response to a delayed cutaneous hypersensitivity reagent while they were undergoing dialysis. Only 17% of 183 dialysis patients responded to skin testing with 2,4 dinitrochlorobenzene (DNCB). Following transplantation, the 1 year allograft survival was significantly poorer in the group of DNBC responders. This approach is only applicable to a small proportion of transplant candidates.

T cell function as measured by PHA and Con A stimulation is reduced in uraemic patients. Thomas et al [1] initially described a correlation between pre-transplant PHA and Con A mitogenesis and early allograft rejection episodes; subsequent studies indicated a lower incidence of irreversible rejection and improved allograft survival in the low responder group [2]. Jones et al [3] indicated that pretransplant PHA responsiveness was predictive of allograft survival. The group of recipients with a low relative response to PHA stimulation had a 54% 6 month survival rate, whereas, the group with a high response had only a 26% survival rate. The relatively poor overall allograft survival results in this report and the short term follow-up as well as the inability of Kerman et al [4,5] to correlate pretransplant PHA responsiveness and allograft survival indicate a need for additional data before PHA responsiveness can be considered predictive of a non-responder status.

A subpopulation of TRFC which is detected by a short incubation period of SRBC and peripheral blood lymphocytes and termed 'active' T cells have been purported to be the T cells actively involved in cellular immunity [16,17]. Kerman et al [4,5] in a preliminary report indicated that lower levels of 'active' T cells pretransplant were associated with improved renal allograft survival, whereas the TRFC levels were not prognostic of allograft outcome.

Our preliminary data suggests that pretransplant TRFC levels are indicative of a non-responder status. The excellent correlation between allograft outcome and low pretransplant TRFC levels is shown by the 94% 18 month survival rate in this group of cadaver donor renal allograft recipients. Validation of our results
from a larger series and other centres is obviously necessary; however, TRFC levels as an indication of the non-responder status may prove useful in clinical renal transplantation.

References

2. Thomas, F, Mendez, G and Picon, J (1977) Transplant Proc., 9, 49
11. Lazarus, JM and Birtch, AG (1973) Transplantation, 15, 568

Open Discussion

KERR (Newcastle) Do you get any better prediction of transplant survival from rosette-forming cell counts than from absolute lymphocyte counts?

UITTENBOGAART I have not really tried to use the absolute lymphocyte count. I have only looked at the T-cell level and in correlation with low, medium and high groups and graft survival.

KERR (Newcastle) I asked the question because one of your slides suggested that the people with low T cells had low T cell counts because they had low absolute lymphocyte counts.

UITTENBOGAART I do not think so.

LEGRAIN (Paris) I will take the opportunity of this very interesting paper to make a general comment. So, it is not really aimed at you.

In recent years many papers, including some presented at this meeting, have been claiming the beneficial effect of some type of selection and/or of some immune treatment, and unfortunately the results of the control group are often very bad.

It has been shown by many groups that with the usual immunosuppression with a medium match without DR-matching, with pretransplant transfusion, a patient survival of 95% at one year and a graft survival of 75% can be obtained. Unless the proposed new means of selection or new treatment improve on these results it is difficult for the clinician to be convinced that they are really clinically useful.