

Natural killer cells in renal allograft rejection

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Monoclonal antibody Anti Leu 7 has been used to enumerate peripheral blood NK cells in 20 renal allograft recipients. Blood was taken pre-operatively and three times per week for the first month after transplantation; separated mononuclear cells were stained with Anti Leu 7 then analysed on a fluorescence activated cell sorter. Results were related to acute rejection episodes diagnosed clinically and confirmed by Trucut biopsy of grafts. A reduction in the number of Leu 7 positive cells occurred postoperatively. Nineteen out of 20 patients showed a rise in number of circulating NK cells during acute rejection episodes.

Keywords: Natural killer cells, renal allograft, acute rejection

Natural killer cells are mononuclear leucocytes which without prior stimulation kill several types of tumour derived or virus infected cultured target cells *in vitro*. They are otherwise negatively defined as non-T, non-B, non-adherent and non-phagocytic cells. They incompletely express T lymphocyte and myelomonocytic surface phenotypes¹.

Morphologically natural killer (NK) cells are a heterogeneous group of cells, a large fraction of which are large granular lymphocytes². These are distinguishable from T lymphocytes by their larger rim of cytoplasm which is stippled with azurophilic granules. Large granular lymphocytes constitute between 5 and 15 per cent of normal human peripheral blood mononuclear cells.

NK cells appear to play a part in allograft rejection. In bone marrow transplantation NK cells are probably major effector cells in natural, radiation resistant rejection³. Increased NK activity has also been associated with clinical graft versus host disease⁴.

In acute rejection of solid organ grafts the role of NK cells is less clear. In murine models up to 10 per cent of mononuclear cells infiltrating rejecting renal allografts are NK cells with high cytolytic activity. Nemlander⁵ has reported that infiltration of rat kidney allografts by NK cells occurs early in the rejection process, before that by cytotoxic T lymphocytes.

In man NK activity is usually measured with a cytotoxicity assay using K562 cell line derived from the pleural effusion of a patient with chronic myeloid leukaemia. Using this assay Guillou *et al.*⁶ have reported an increase in NK activity to be associated with episodes of acute rejection in 12 patients. These authors did, however, suggest that the considerable day-to-day variation in the susceptibility of target cells to lysis makes serial estimations of this kind difficult to interpret.

Two monoclonal antibodies Anti Leu 7⁷ and Anti Leu 11⁸ are now available which have been developed to bind to surface antigens specific to NK cells. Anti Leu 7 binds to large granular lymphocytes and Anti Leu 11 to the majority of cells exhibiting natural killer activity.

The aim of this study was to enumerate NK cells in the peripheral blood of human renal allograft recipients using Anti Leu 7 and to relate these results to episodes of acute rejection.

Materials and methods

Twenty patients who received cadaveric renal allografts in the Nottingham unit during 1983 have been studied. Pre-operatively all patients received at least three blood transfusions. The minimum tissue typing match was 1 Dr identity.

Maintenance immunosuppression consisted of 20 mg prednisolone from the day of transplantation plus azathioprine 2 mg/kg daily. The treatment of rejection episodes consisted of a 12 day reducing course of oral prednisolone starting at 200 mg per day reduced by 20 mg every 3 days.

Ten millilitres of venous blood was taken pre-operatively and then three times each week for the first month after transplantation.

Mononuclear cells were separated from fresh heparinized blood using a Ficoll hypaque density gradient technique modified from

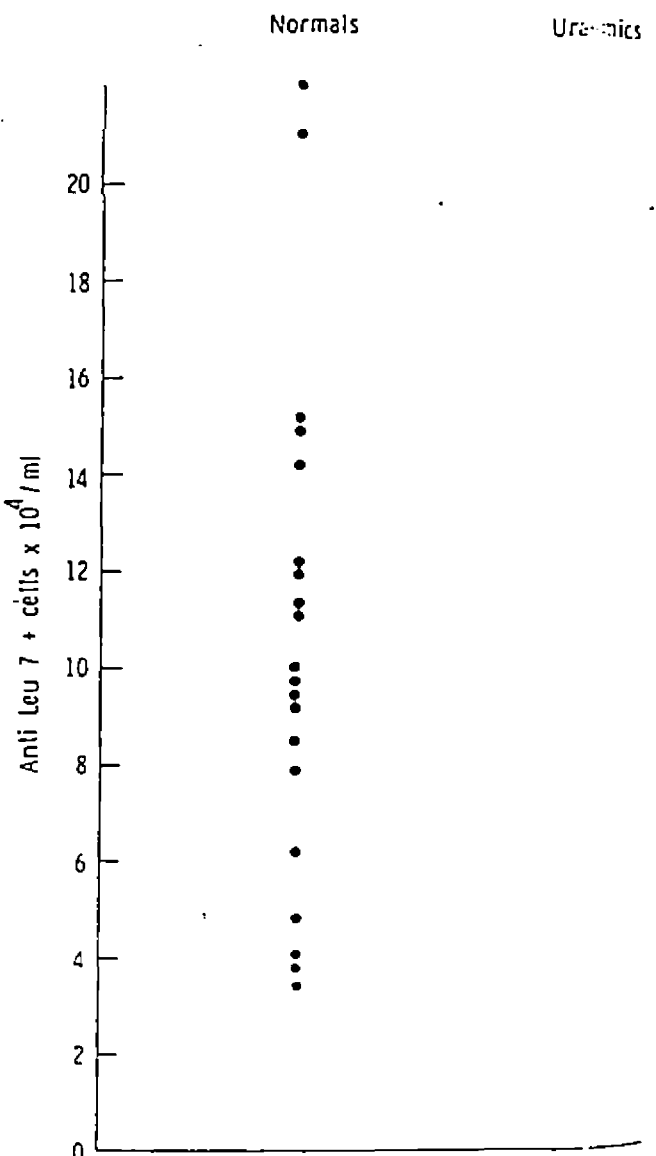


Figure 1 Number of Leu 7 positive cells $\times 10^4$ /ml blood in 20 patients before transplantation compared with 20 age and sex matched normals.

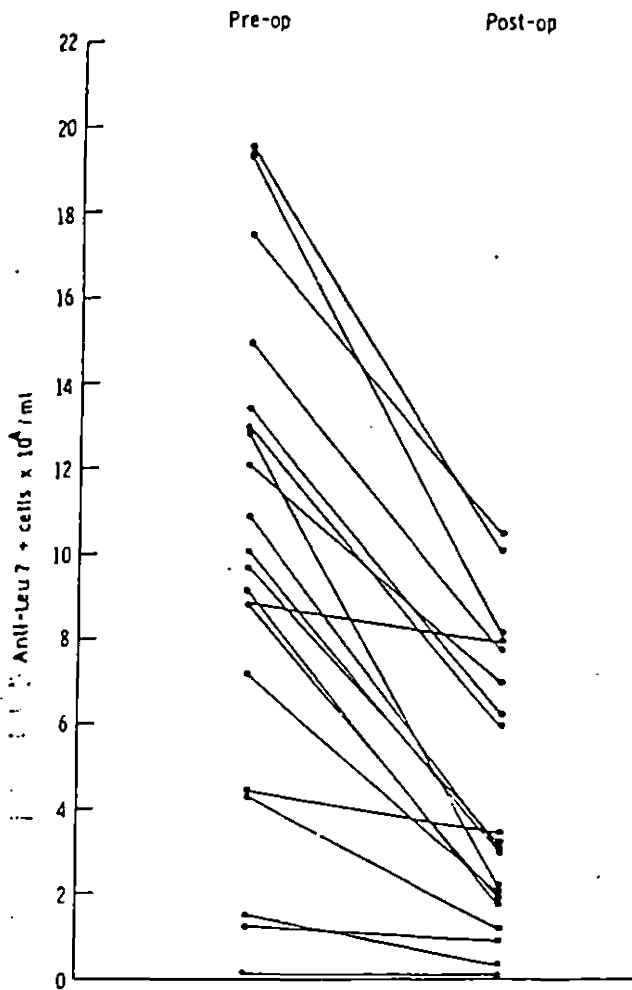


Figure 2 Number of Leu 7 positive cells $\times 10^4$ ml blood before and after transplantation. Student's *t* test, $t = 7.2265$; $P < 0.001$

Boyum¹¹. Between 2.5 and 5×10^5 washed cells were then labelled with Anti Leu 7 using an indirect fluorescent technique. The percentage of lymphocytes labelled with Anti Leu 7 was determined using a fluorescence activated cell sorter utilizing forward angle and 90° scatter gates to confine the analysis to lymphocytes (FACS IV, Becton Dickinson). The total number of cells per millilitre of blood was calculated from this percentage, a white blood cell and a differential count from the same blood sample.

Results have been related to episodes of acute rejection which were initially diagnosed on the conventional clinical and biochemical criteria of: graft swelling and tenderness; fluid retention; decreased urine output; hypertension; fever in the absence of infection and elevation of serum creatinine. In all cases a Trucut needle biopsy was performed, before the start of treatment for acute rejection, for histological confirmation of the diagnosis. Technical and infective causes of graft failure were excluded by routine isotope scanning of allografts, urine and blood culture and viral serology. All rejection episodes documented received treatment, as outlined above, which on review we feel was justified in each case.

Results

The number of Leu 7 positive cells per millilitre of blood before transplantation in our 20 patients and in 20 age and sex matched normal volunteers is shown in Figure 1. There was no significant difference between these two groups. Student's $t = 0.543$; $P > 0.5$.

In the first 3 days after transplantation there was a reduction in the number of Leu 7 positive cells in all patients as shown in Figure 2. Student's paired $t = 7.23$; $P < 0.001$.

Figure 3 represents the number of Leu 7 positive cells and creatinine levels of a typical patient, who received treatment for rejection on the tenth postoperative day. The previous day a rise in Leu 7 positive cells greater than two standard deviations above the mean of three postoperative values occurred along with a modest rise in serum creatinine level.

In the 48 h before the start of treatment for rejection we have observed a rise in both the percentage of lymphocytes positive for Anti Leu 7 and in the actual number of such cells per millilitre of peripheral blood in 19 out of 20 patients as compared with previous samples (see Figure 4). Student's paired $t = 3.768$; $0.01 > P > 0.001$.

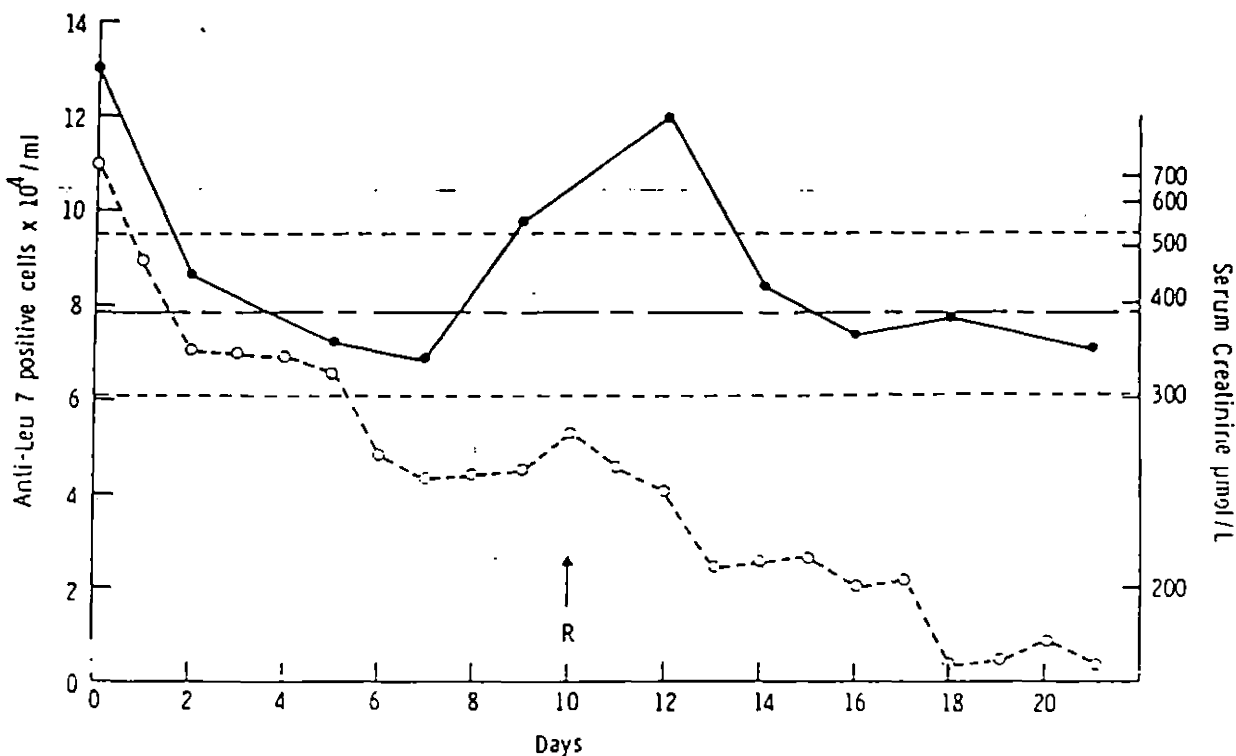


Figure 3 Number of Leu 7 positive cells (continuous line) and serum creatinine levels (broken line) of one patient during the first three weeks after

In 17 patients there were three or more postoperative but pre-rejection values from which to derive a mean and standard deviation. In 12 cases the increase in Leu 7 positive cells at the onset of rejection was greater than two standard deviations above the mean, in four cases there was a rise which was less than two standard deviations and in one patient numbers fell.

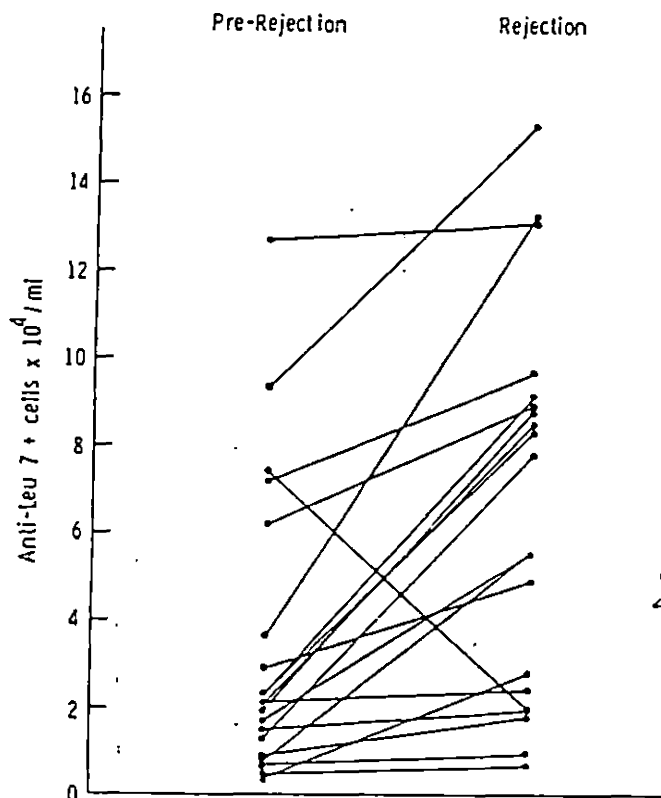


Figure 4 Number of Leu 7 positive cells $\times 10^4$ /ml blood before and at the onset of acute rejection episodes. Student's paired *t* test, $t = 3.768$; $0.01 > P > 0.001$

Following the start of treatment for rejection both percentage and actual numbers of Leu 7 positive cells promptly fell to pre-rejection levels. This fall was observed in both rejection episodes which were successfully treated and those three patients where treatment was unsuccessful and graft failure ensued.

Four patients had only small rises in numbers of Leu 7 positive cells at the onset of acute rejection episodes. These patients all had low cell numbers in the pre- and postoperative samples when compared with the other patients.

It is of interest that the one patient whose cell numbers fell at the onset of acute rejection (and before the start of treatment) was one of the three patients to lose graft function during the period of study.

One patient also investigated during the period of study was not included in the above analysis as no acute rejection has occurred in over 1 year of follow-up. Figure 5 illustrates that the number of circulating Leu 7 positive cells remained at the post-transplant level for the whole 3 weeks in this patient.

Discussion

Monoclonal antibodies in conjunction with FACS analysis are rapid and convenient probes with which to investigate natural killer cells in a variety of situations. The reproducibility of this method makes it most suitable for the examination of serial samples as in this study (whereas the variability of the ^{51}Cr cytotoxicity assay mentioned previously makes results difficult to interpret in this situation).

It is acknowledged that Anti Leu 7 does not label all circulating mononuclear cells with NK activity and that overlap with cells of suppressor phenotype does exist. However, in a previous report of Helper and suppressor cells in this same group of patients we have found no increase in Leu 2a positive suppressor cells to occur at the time of rejection¹⁰ which could account for our findings.

There are several factors which may explain the observed fall in number of Leu 7 positive cells after transplantation. Surgical stress is known to alter immune-competence; in particular lymphocyte responses to mitogenic stimuli are well known to fall after a variety of surgical procedures¹¹. In addition, corticosteroids are known to depress NK activity. Lenn *et al.*

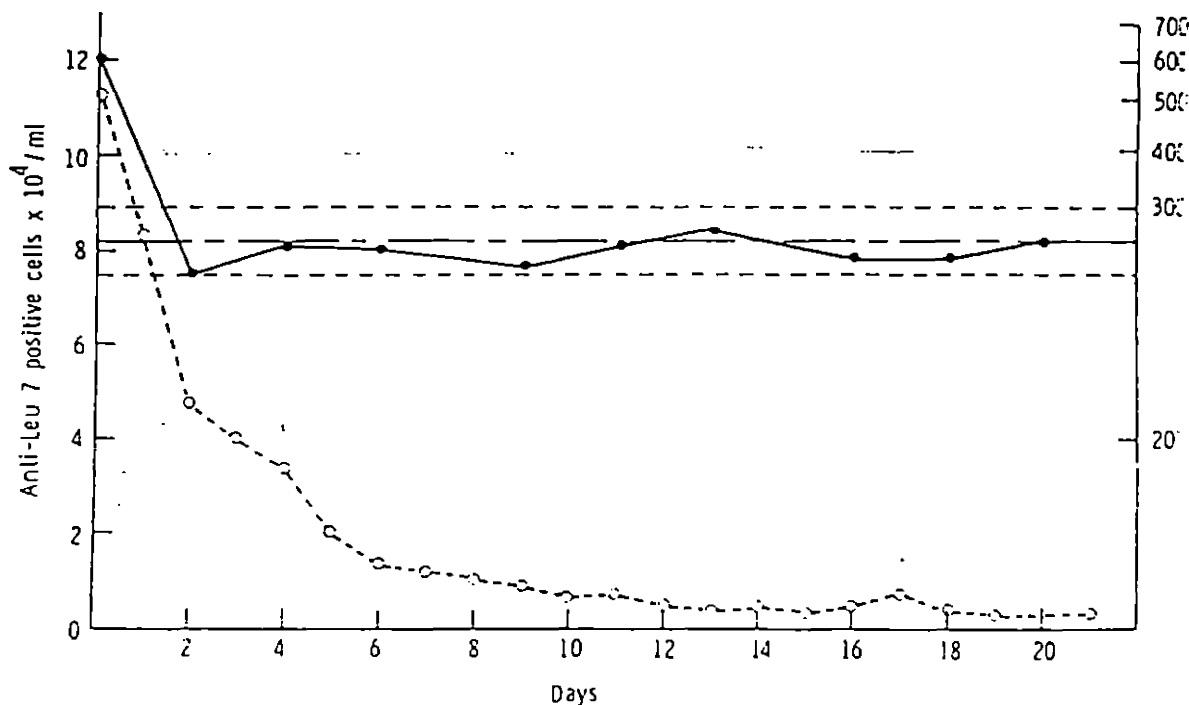


Figure 5 Number of Leu 7 positive cells $\times 10^4$ /ml blood and serum creatinine levels of one patient during the first three weeks after transplantation

(personal communication) have observed a fall in number of circulating Leu 7 positive cells to follow uncomplicated surgery such as cholecystectomy and femoropopliteal bypass. However, in these cases numbers returned to pre-operative levels within 7 days.

In studies of this kind the definition of acute rejection is a difficult problem and a potential pitfall. The histological features of allografts seen in Trucut needle biopsies is not a gold standard which can be used alone. In this study the diagnosis of each acute rejection episode was reviewed by an experienced clinician who assessed all the clinical and histological findings together. In the majority of cases presented, acute rejection episodes were clear cut events. This is due to the rather aggressive nature of rejection episodes seen in the first month after transplantation where low doses of prednisolone are used for maintenance immunosuppression.

In this study we have found a significant increase in circulating NK cells, as defined by Anti Leu 7, to occur in renal allograft recipients at the onset of acute rejection episodes. While the role of NK cells in renal allograft rejection remains unresolved, our findings add to the increasing body of evidence which suggests that these cells are involved in rejection. The question remains as to whether this rise in NK cells represents the activation of an effector pathway actively involved in the rejection process or whether it is a non-specific finding reflecting activation of the immune system. Recent data¹² which has shown NK activity to be augmented by Inter-leukin 2 points to a regulatory role for NK cells in the immune network. Thus studies of the mechanism of acute rejection now need to consider the involvement of NK cells in this process.

The conclusion of this study is that the number of circulating natural killer cells as defined by the monoclonal antibody Anti Leu 7 increases in the majority of renal allograft recipients undergoing acute rejection.

Acknowledgements

We thank John Lawry and Owen Roberts for their considerable help with FACS analysis of our specimens.

R. A. Robins is supported by the Cancer Research Campaign.

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Paper accepted 15 August 1984