MONITORING OF NK-1 AND NK-15 SUBSETS AND NATURAL KILLER ACTIVITY IN KIDNEY TRANSPLANT RECIPIENTS RECEIVING CYCLOSPORINE

E Renna Molajoni, V Barnaba, A Bachetoni, M Levrero, P Cinti, M Rossi, D Alfani, R Cortesini

University of Rome, Rome, Italy

Summary
NK-1, NK-15 subsets and natural killer (NK) activity were studied in 13 patients, before undergoing renal transplantation and in the post-transplant period. Pre-transplant there was a significant reduction of NK activity with a defect in V_max capacity in all patients. NK-1+ and NK-15+ cell number was normal or slightly increased in all subjects.

Post-transplant evaluation under cyclosporine immunosuppression showed a quantitative reduction in NK cells while no changes in NK activity and killing activity have been seen.

We conclude that in spite of a numerical decrease, NK activity was not affected by cyclosporine treatment.

Introduction
The main goals in organ transplantation are to avoid the two major complications of rejection and infection. Over the last twenty years several drugs have been employed to control recipient immunological reactivity against HLA alloantigens [1]. None of these methods have shown a specific and/or selective effect on immunocompetent cells involved in the rejection mechanisms [2]. Recently cyclosporine employed as immunosuppressive therapy in different organ allografts, has demonstrated a selective activity on helper cells and IL-2 release without affecting other subpopulations which play an important role against viral, bacterial and fungal infections [3]. On this basis, we have studied NK cells number and function in patients who have undergone renal transplantation, and we have examined the relationship between NK activity and clinical outcome.

Material and methods

Patient population and control

Thirteen patients ranging in age from 18 to 52 years have been studied before transplantation and 10 of these cases, periodically, in the post-operative period.
Seven patients received transplants from living related donors and three from living unrelated donors. All patients were transplanted with ABO blood group compatibility, with two or more mismatched HLA antigens and a negative cross-match.

Twenty healthy subjects matched for age and sex were used as controls.

Cyclosporine therapy

Cyclosporine was given as pre-transplant treatment at a dosage of 5mg/kg/day IV for 3–5 days before transplantation and in the post-transplant period according to the following protocol:

- 5mg/kg/day IV until oral adsorption is restored
- 15mg/kg/day from the end of IV treatment to day 15
- subsequent decrease of the dose by 2mg/kg/month until a maintenance dosage of 2–6mg/kg/day has been reached.

Lymphocyte-surface markers studies

NK-1 and NK-15 subsets were measured using murine monoclonal antibodies Leu 7 and Leu 11a (Becton Dickinson) in an indirect immunofluorescence assay [4].

Target cells and culture medium

All target cells were cultured at 37°C in a humified air atmosphere with five per cent CO₂ in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10 per cent fetal calf serum (FCS GIBCO) and 100µg/ml gentamicin. K562, a human tumour cell line derived from a patient with chronic myelogenous leukaemia, and MOLT-4, a human T cell leukaemia derived line, were used as target cells. Results obtained with the MOLT-4 line were comparable to those observed with K562 and therefore we have only reported data with K562 target cells.

Effector cells

Peripheral blood lymphocytes (PBL) were isolated from heparinised venous blood by separation on Ficoll-Hypaque gradients [5].

Toxicity assay

Cytotoxicity tests were performed by a standard ⁵¹Cr-release assay in standard 96-well U-shaped microplates (Falcon Plastics) in a total volume of 200µL. One million target cells were labelled with 200µCiNa₂ ⁵¹CrO₄ (Sorin, Saluggi, Italy) for one hour at 37°C. Ten thousand labelled target cells (100µL) were mixed with varying numbers of effector cells (100µL) to give final effector: target (E:T) ratios ranging from 50:1 to 6:1.
After four hours incubation period, 100μL of supernatant were collected and cytotoxicity was estimated from released radioactivity according to the formula:

\[
\text{Per cent of specific cytotoxicity} = 100 \times \frac{\text{cpm exp} - \text{cpm } s_r}{\text{cpm } m_r - \text{cpm } s_r}
\]

where cpm exp equalled the mean of the observed quadruplicate assay, cpm s_r was spontaneous release and cpm m_r was maximal release achieved by treating target cells with Nonidet P40. Spontaneous release of ⁵¹Cr by target cells was determined by placing labelled target cells in microtitre wells in the absence of effector cells.

**Natural killer (NK) capacity (V_max)**

The V_max of the NK assay represents the maximum number of target cells that can be killed by a constant number of effector cells when target cells are present in excess. V_max was determined according to the method of Ulberg and Jondal [6] with minor modifications. Briefly target cells were labelled with ⁵¹Cr as described above. After washing, dilutions of target cells at five different concentrations were prepared (varying from 0.25 x 10⁵ to 0.015 x 10⁶ cells). To each well were added 10⁵ effector cells and the cell mixture (0.2ml) was incubated for three hours at 37°C in five per cent CO₂. For each E:T ratio the maximal spontaneous lysis was determined.

The dose-response curve from the ⁵¹Cr release cytotoxicity assay can be expressed as:

\[
V = \frac{V_{\text{max}} \times T}{K_m + T}
\]

where T is the initial number of target cells, V the number of killed target cells and V_max the number of target killed cells when T approaches infinity, that is when the system is saturated with target cells. Km is the number of target cells that produces one-half of V_max. V_max and Km can be calculated using the lineweaver-Burk equation:

\[
\frac{1}{V} = \frac{K_m}{V_{\text{max}}} \times \frac{1}{T} + \frac{1}{V_{\text{max}}}
\]

In this equation there is a linear relationship between 1/V_max and 1/T. The reciprocal values of V and T can be plotted and regression analysis used to determine V_max from the reciprocal of the Y intercept and X intercept respectively.
Results

Pre-transplant period

Pre-transplant study showed a significant reduction of NK activity, especially at lower ratios with a defect in $V_{\text{max}}$ capacity in all patients. NK-1+ve and NK-15+ve cell number was normal or slightly increased in all patients (Table I).

<table>
<thead>
<tr>
<th>NK Subsets</th>
<th>Control Group</th>
<th>Pre-transplant</th>
<th>Post transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK1</td>
<td>16.5±3.0</td>
<td>17.6±2.1</td>
<td>8.6±1.9</td>
</tr>
<tr>
<td>NK15</td>
<td>16.1±2.5</td>
<td>18.2±3.7</td>
<td>10.4±2.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NK Activity</th>
<th>E:T</th>
<th>$V_{\text{max}}$ Killing capacity (x $10^5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50:1</td>
<td>29.9±2.9 (p&lt;0.02)</td>
<td>8.3±5.2 (p&lt;0.001)</td>
</tr>
<tr>
<td>25:1</td>
<td>24.4±2.9 (p&lt;0.005)</td>
<td>20.1±2.2 (p&lt;0.02)</td>
</tr>
<tr>
<td>6.1</td>
<td>17.4±1.5 (p&lt;0.001)</td>
<td>15.1±1.9 (p&lt;0.005)</td>
</tr>
</tbody>
</table>

Post-transplant period

Post-transplant evaluation under cyclosporine immunosuppression showed a quantitative reduction of NK cells, while no changes in NK activity and killing capacity ($V_{\text{max}}$) were seen.

Discussion

In recent years, NK activity has been considered an important effector mechanism in host defence against tumours and virus infected cells [7]. Lack of NK activity is associated with infection and a significant impairment of cellular immunity has been observed in subjects affected by uraemia [8]. This impairment is worsened by conventional therapy with steroids, azathioprine and/or antilymphocyte globulin-employed in the post-operative period in patients who undergo organ allografts.

Cyclosporine seems to exert a more selective activity, as demonstrated by in vitro and in vivo studies [9]. Our studies on NK cells in transplanted patients receiving cyclosporine confirms this hypothesis. Even though a slight numerical decrease of NK-1 and NK-15 cells has been observed in all the cases, no changes in NK activity and killing capacity have been seen. The low incidence of infective events support these results with only one out of 10 patients developing CMV infection.

Finally, the decrease of NK-1 and NK-15 cell subsets, in spite of an unmodified
NK function, indicate that NK quantitation by monoclonal antibody assays is not of great value in post-transplant monitoring, particularly concerning immuno-competent cells involved against infections.

Acknowledgment

This work was supported in part by a grant from ‘Institute Pasteur Fondazione’ Cenci Bolognetti.

References

1 Renna Molajoni E, Sansonetti P, Nesci C et al. Proc EDTA 1977; 14: 495
3 White DJG. Cyclosporine. Amsterdam: Elsevier Biomedical. 1982: 578
5 Boyum A. Tissue Antigens 1974; 4: 269