SUPPRESSOR CELL ACTIVITY IN HUMAN RENAL ALLOGRAFT RECIPIENTS

C Hammer, W Land, L A Castro

Institute for Surgical Research and Transplant Centre of the Surgical Clinic, University of Munich, FRG

Summary

Eighty-nine kidney allograft recipients with one or more graft rejection episodes were monitored for suppressor cell activity in the peripheral blood at two day intervals. The impact of rejection and immunosuppressive therapy (ALG) on the one hand and the recovery of suppressor activity on the other hand during long-term graft survival could be demonstrated.

The suppressor activity of peripheral lymphocytes after amplification in vitro by Concanavalin A was measured on PHA stimulated cultures. It is reduced after operation trauma and during rejection episodes when tested in PHA stimulated cultures.

This loss of suppressor activity is even more marked when suppressor cells are added to allogeneic mixed lymphocyte reactions. Depletion of adherent cells reduces this suppressor activity, indicating that two different populations of mononuclear cells are responsible for this in vitro effect.

Immunosuppressive therapy and additional ALG treatment abolishes suppressor activity in most cases. Highest suppressor activity was monitored in seven patients with excellent long term graft survival after ALG therapy.

Introduction

Subsets of peripheral lymphocytes display suppressive activity which has been found to regulate the homeostasis of the normal immune response. Persistent or chronic stimulation, however, can cause disordered immune regulation, leading to high suppressor cell activity. In human renal allograft recipients amplification of this function could be beneficial for the patient. It could therefore be important, not only to monitor the patients' immunological reaction against the graft, but also the short and long-term effect of the various immunosuppressive regimens on the regulator cell population. This is possible by enhancing suppressor cell activity in the peripheral blood of the patients by exposure to polyclonal mitogens such as Concanavalin A or Phytohaemagglutinin. This treatment leads not only to
the induction of nonspecific suppressor cells, but possibly to further amplification
of the mature antigen-specific suppressor cells.

Since both mature and precursor suppressor cells circulate in peripheral blood
they are exposed directly to immunosuppressive therapy. Thus the impact of
potent but nonspecific immunosuppressants, like ALG, could have a deleterious
effect on the suppressor cells.

Material and methods

Peripheral blood mononuclear cells of the recipient, of the related donor or of
unrelated healthy control persons were purified by centrifugation of 1:1 diluted
venous blood on Ficoll Hypaque gradients.

To avoid circadian influences the blood was drawn only in the morning.

Cells in the interphase ring were washed twice in RPMI 1640 and resuspended
in RPMI enriched with 10 per cent heat inactivated fetal calf serum, 100 units/ml
penicillin, 100µg/ml streptomycin and 2mM L-glutamin. Cell viability was deter-
mined by trypan blue dye exclusion and the cells adjusted to a final concentra-
tion of 1 × 10⁶/viable cells per ml.

Half of each patient’s cells were cultured over a two day period at 37°C, in 5
per cent CO₂ with and without 20µg/ml Concanavalin A. After two days the cells
were washed in RPMI, irradiated with 1600 rads in a Caesium-γ-source and adjusted
to 1 × 10⁶/ml viable cells in enriched RPMI medium. Those cells stimulated by
Con A are called ‘activated suppressor cells’, those cultured without Con A are
termed ‘spontaneous suppressor cells’.

Two tests were used as indicator systems, (a) the PHA stimulated culture and
(b) the one way mixed lymphocyte reaction:

(a) Either 0.1ml (1 × 10⁵) of activated spontaneous suppressor cells were added
to freshly prepared cultures of 2 × 10⁵ PHA stimulated lymphocytes (2µg/ml) in
round bottomed culture plates. After three days of cocultivation of indicator and
suppressor cell populations, 2µci of ³H-thymidine were added and the cultures
harvested after a further 18 hours of incubation.

(b) 0.1ml containing 1 × 10⁵ lymphocytes were plated on to round bottom
plates as either responder, stimulator or regulator i.e. suppressor cells in a ratio
of 1:1:1. For the MLR, stimulator cells were irradiated with 1600 rads as well as
the regulator cells from the patient when introduced as suppressor cells. All cul-
tures were set up in triplicate, MLRs were incubated for six days at 37°C in a
humidified 5 per cent CO₂ atmosphere. For the last 18 hours of incubation 2µci
of ³H-thymidine were added to each well.

Like the mitogen stimulated cultures those allogeneic MLRs were frozen and
then collected with a multiple sample harvester. Incorporation of ³H-thymidine
into the nuclear DNA was measured in a liquid scintillation counter.

When run as an autologous suppressor assay, the responder and suppressor
cells were from the same patient, stimulating cells were either from the donor
(spleen) or a normal third party donor. In the allogeneic suppressor assay only
the suppressor cells were from the transplant patient. Third party cells were taken
from one person throughout the test.
Suppression was calculated by the formula:

\[ \text{Suppressor activity in } \% = \left( \frac{\text{cpm of } 1 \times 10^5 \text{ suppressor cells irradiated} + 1 \times 10^5 \text{ indicator cells (PHA/MLR)}}{\text{cpm of indicator cells (PHA/MLR)}} \right) \times 100 \]

This suppressor test was performed in 89 patients at two to three day intervals as long as the patients were hospitalised or came for outpatient study.

All 89 patients were treated according to the following schedule:
In the first week prednisolone was given in decreasing amounts from 100mg to 60mg/day, azathioprine was reduced from 5mg/kg BW at day 1 to 2.5mg/kg at day 6. Commercial horse anti-human lymphocyte globulin from Behringwerke, the same good batch available in reasonable amounts, was ultracentrifuged for two hours in order to eliminate aggregates and 20mg of protein were given per kg BW intravenously as an infusion in 250ml of saline over a period of 10 days starting with the first postoperative day. Reduction of the drugs continued to reach the maintenance therapy of 15mg prednisolone and 1.5mg/kg azathioprine per day after approximately six months. During rejection episodes methylprednisolone was increased as well as ALG (40mg/kg BW) over another 10 days.

Results

Suppressor activity measured preoperatively in eight patients showed almost identical inhibition of both PHA stimulated cultures and MLRs (Table I). Here spontaneous suppressor cells reduced the proliferation of indicator cultures by 29 and 24 per cent and activated suppressor cells by 39 and 41 per cent, respectively. These are values almost identical to controls. Post transplantation and with the start of therapy lymphocyte counts dropped to 40 per cent of the initial values.

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>MLR</th>
<th>PHA</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Spontaneous suppressor cell activity</td>
</tr>
<tr>
<td>Pre-operative</td>
<td>8</td>
<td>-24%</td>
</tr>
<tr>
<td>Post transplant</td>
<td>15</td>
<td>+17%</td>
</tr>
<tr>
<td>ALG treatment</td>
<td>12</td>
<td>+1%</td>
</tr>
<tr>
<td>1st rejection</td>
<td>22</td>
<td>+16%</td>
</tr>
<tr>
<td>2nd rejection</td>
<td>13</td>
<td>+7%</td>
</tr>
<tr>
<td>1st rejection + ALG</td>
<td>12</td>
<td>+30%</td>
</tr>
<tr>
<td>Long graft survival</td>
<td>7</td>
<td>-37%</td>
</tr>
</tbody>
</table>

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As B cells remain relatively constant, T cells show major reduction. The suppressive activity of lymphocytes in mitogen-stimulated cultures is reduced by both activated and spontaneous suppressor cells by around 40 per cent. Suppression of allogeneic MLRs decreases, resulting in many cases in further stimulation of the cultures. Additional ALG treatment of the patients seems to have a more pronounced effect on spontaneous suppressor cells, while the function of activated cells remains constant.

Massive impact on suppressive activity in the allogeneic cultures was observed during acute rejection episodes. Neither activated nor spontaneous suppressor cells showed activity in allogeneic MLRs. There was no major difference in suppression as compared with the autologous HLA donor specific tests. Lymphocytes from patients who underwent repeated rejections initiated enhancement of the in vitro cultures rather than suppression. Opposite results were found on PHA-stimulated cultures where suppressor activity reached peak values increasing during repeated rejection episodes. When ALG (40mg/kg BW) was added during rejection, the function of spontaneous suppressor cells was abolished as before, leading once more to stimulation of the indicator cultures. These lymphocytes can still be activated again by Concanavalin A to the postoperative suppressor values.

In seven patients displaying almost no signs of rejection for more than 18 months, suppressor activity reached values exceeding those preoperatively by around 50 per cent.

Discussion

The data show that suppressive activity of peripheral mononuclear cells from patients with kidney grafts can be detected in vitro [1]. This activity is reduced not only by immunosuppressive therapy but also by operation and rejection [2]. In some cases suppression is even converted into stimulation. This happens especially during massive immunosuppression or after repeated rejections.

ALG used during rejection reduces circulating cells and eliminates mainly the spontaneous suppressor activity which is supposed to be more antigen specific [3]. This would explain the loss of suppressive activity in MLR, but not in mitogen stimulated cultures. It also suggests that those circulating lymphocytes which are amplified by specific antigens are eliminated first by immunosuppression, especially ALG. The suppressor precursor cells not yet induced seem to be less sensitive to the treatment and can therefore be activated by Concanavalin A in vitro. This Con A-induced suppressor activity is less specific, since their function is better expressed when tested on mitogen-induced proliferation [4]. During the onset of rejection suppressor activity decreases; again more pronouncedly in allogeneic test systems. One cannot exclude the possibility that the complete loss during repeated rejection episodes is due to the impact of the increased immunosuppressive therapy. The good suppressor activity displayed at the same time in mitogen cultures again suggests that two populations of suppressor cells [5] are influenced during rejection. Whether this correlates with the finding that depletion of either monocytes or T cells reduces suppressor activity [6] could not so far be tested.
The high suppressive activity in long-term graft survivors supports the hypothesis that chronic antigenic stimulation increases suppressive function [7]. The identical values before transplantation and during good function when only low immunosuppressive therapy is required, indicates that homeostasis can be reached again at a higher 'suppressor tone' which might be beneficial for the transplanted patient.

Acknowledgment

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References

1 Jaravan M, Braun WE, Novick A. Transplant Proc 1979; 11: 4
3 Wood ML, Gottschalk R, Monaco AP. Transplantation 1979; 28: 5
5 Raff HV, Cochrum KC, Stobo JD. Transplantation 1978; 121: 6

Open Discussion

VAN YPERSELE (Chairman) Have you monitored the suppressive activity during the evolution of rejection, when your graft was recovering? Were there any changes to be seen accompanying the recovery?

LAND We did some studies not shown in this paper following the suppressor activity after the onset of ALG. Those studies showed that the suppressive activity recovered approximately 14 days after the offset of ALG. We might conclude from those studies that during ALG therapy we kill the cytotoxic cells plus suppressor cells, but after discontinuing ALG the suppressor cells return earlier or better than the cytotoxic cells. That is the only study we have done in this respect.

BARNES (Birmingham, United Kingdom) In your long-term transplanted patients did you have any that weren’t in your high and very high group, were there some of them that were down in the lower group? These were average figures that you were showing, were there any that didn’t quite go along with it. Was it absolutely clear-cut?

LAND Yes, in those long-term studies it was always the same result.