IMPROVEMENT OF CADAVERIC RENAL ALLOGRAFT SURVIVAL BY THORACIC DUCT DRAINAGE: RELATION WITH T-LYMPHOCYTE SUBSET MODIFICATIONS ASSESSED BY FLOW-CYTOMETRY

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Summary

Thoracic duct drainage (TDD) with reinfusion of cell-free lymph was performed in 118 prospective recipients within four months before transplantation. TDD was unsuccessful in 27 patients (A); it yielded \(1 - 19 \times 10^9\) lymphocytes in 25 cases (B) and \(20 - 185 \times 10^9\) in 66 cases (C). The incidence of acute rejection episodes and the requirement for early post-transplant dialysis were lower in C than in A.

Six patients were studied for T-lymphocyte subsets, using monoclonal antibodies from OKT series and a monomorphic and HLA-DR (BL2) raised in our laboratory. During TDD peripheral blood lymphocyte (PBL) counts decreased and the percentage of BL2 cells increased. Simultaneously, typical small PBL were replaced by large less differentiated cells slightly labelled by OKT3, some of them bearing both OKT4 and OKT8 markers. The larger the depletion, the earlier the emergence of immature T-cells. In lymph fluid, lymphocyte counts decreased later than in blood, the proportion of T8+ cells lacking Fc receptors increased with time. Changes in B patients were less than in C. These results support the hypothesis that T-cell subset modifications represent the main immunological change accounting for better allograft prognosis.

Introduction

The immunosuppressive effect of thoracic duct drainage (TDD) has long been studied, either in animal experiments [1] or in a wide spectrum of clinical situations, including organ transplantation [2–6] and autoimmune diseases [7,8]. However, in kidney transplantation, the favourable results reported after TDD have not always been clearly established by comparison with appropriate control series. In humans, unlike rodents, TDD is not supposed to achieve a massive depletion of the recirculating lymphocyte populations, but rather to induce modifications of lymphocyte subsets demonstrable by their surface markers and
proliferative responses [9–11]. Our aim was to study kidney allograft survival in patients submitted to pre-transplant TDD, and differing only by the efficiency of the TDD, and in the induced modifications of lymphocyte subsets enumerated using flow-cytometry and monoclonal antibodies.

Patients and methods

Thoracic duct drainage

Over the past six years, 118 kidney allograft recipients underwent pre-transplant TDD. Surgical access to the terminal part of TD was performed by a left subclavicular incision, as previously described [12]. The TD was then cannulated by a silastic catheter, recently modified to continuously infuse a heparin solution in order to prevent lymph fluid clotting. The collected lymph was centrifuged (3,000 rpm, 15 min), and the cell-free supernatant was reinfused after bacteriological controls. Supportive treatments included a diet enriched in long chain triglycerides, and parenteral compensation of fluid losses by a central venous line. At the end of the second week, the drainage was stopped by progressive clamping of the catheter which was then removed.

Lymphocyte subsets analysis

In six patients among the more recent transplant recipients, blood and lymph samples were taken at least twice a week during TDD, blood was then sampled every week until transplantation. Mononuclear cells were separated over a Lymphoprep layer as previously described [13] and resuspended in phosphate buffered saline. The cells were identified by indirect immunofluorescence using monoclonal antibodies from the OKT series (OKT3, OKT4, OKT8, Ortho Pharmaceuticals, Raritan, NJ) and a monomorphic anti HLA-DR (BL2) raised in our laboratory. Labelled cells were then processed on a 50H Cytofluorograf (Ortho Instruments) with an argon-laser (wave length: 488nm). Data were recorded on a computer memory (MINC 11–23 Digital). This disposition gives facilities to identify mononuclear cells by their light-scattering properties and to distinguish between small lymphocytes and large mononuclear cells.

Results

Clinical events during TDD

Bacterial contamination of the lymph fluid or of the catheter were the main complications of TDD, encountered in 37 per cent of all the patients, from whom 13 per cent developed septicaemia. The bacteria predominantly found were Staph epidermidis or Staph aureus, antimicrobial chemotherapy was systematically completed by premature removal of the catheter. Other medical complications included hypovolaemia (which resulted in thrombosis of the arteriovenous fistula in about 10 per cent of the patients), and abdominal pains at the time of clamping the catheter. They were related to hypertension in the lymphatic abdominal system, and may have contributed to acute pancreatitis in five patients. In four patients, a reimplantation of the TD into the external or the internal jugular veins was performed.
**TDD efficiency**

According to the previous data from our group [6], the criteria for a successful TDD were defined as follows: time of effective drainage above five days, and total number of harvested cells above $20 \times 10^9$. Catheterisation of TD failed in 27 patients (22.9%) defined as group A. Among TDD patients, 25 (21.2%) underwent an insufficient drainage (group B), and 66 patients (55.9%) a successful TDD (group C). In group B, the mean duration of the drainage was five days, providing a mean volume of five litres and a mean lymphocyte number of $2 \times 10^9$ cells. Corresponding values in group C were respectively 15 days, 29 litres and $60 \times 10^9$ cells.

**Lymphocyte subset modifications**

During the drainage, a profound lymphopenia developed, with PBL counts as low as 40% of initial values about the eleventh day (Table I). Immunofluorescence on PBL showed, for each patient, extensive changes among the T-lymphocyte subsets, in the form of successive waves during the TDD. They resulted in a marked decrease of OKT3$^+$ and OKT4$^+$ cells, a slight decrease of OKT8$^+$ cells, and a slight increase of BL2$^+$ cells (Table I). Decrease in small

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Source of cells</th>
<th>Day 0 Predrainage</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 11</th>
<th>Day 15</th>
<th>Day 21</th>
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<tbody>
<tr>
<td>OKT3</td>
<td>Blood</td>
<td>68.9</td>
<td>45.5</td>
<td>52.8</td>
<td>43.3</td>
<td>59.8</td>
<td>37.5</td>
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<tr>
<td>OKT4</td>
<td>Lymph</td>
<td>80.0</td>
<td>76.8</td>
<td>73.8</td>
<td>55.3</td>
<td></td>
<td></td>
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<tr>
<td>OKT4</td>
<td>Blood</td>
<td>42.7</td>
<td>30.1</td>
<td>40.5</td>
<td>31.7</td>
<td>36.1</td>
<td>23.6</td>
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<tr>
<td>OKT8</td>
<td>Lymph</td>
<td>63.5</td>
<td>60.3</td>
<td>57.9</td>
<td>49.3</td>
<td></td>
<td></td>
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<tr>
<td>OKT8</td>
<td>Blood</td>
<td>35.2</td>
<td>29.3</td>
<td>28.9</td>
<td>23.4</td>
<td>32.4</td>
<td>26.8</td>
</tr>
<tr>
<td>BL2</td>
<td>Lymph</td>
<td>26.9</td>
<td>28.8</td>
<td>30.2</td>
<td>42.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL2</td>
<td>Blood</td>
<td>21.5</td>
<td>14.5</td>
<td>19.0</td>
<td>20.8</td>
<td>22.2</td>
<td>19.9</td>
</tr>
<tr>
<td>Blood (OKT4 + OKT8 - OKT3)</td>
<td>Lymph</td>
<td>9.0</td>
<td>13.9</td>
<td>16.6</td>
<td>11.8</td>
<td>8.7</td>
<td>12.9</td>
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<tr>
<td>PBL counts</td>
<td></td>
<td>1732</td>
<td>908</td>
<td>776</td>
<td>769</td>
<td>851</td>
<td></td>
</tr>
<tr>
<td>Lymph cell counts</td>
<td></td>
<td>$2.0 \times 10^9$/ml</td>
<td>1.2</td>
<td>1.2</td>
<td>0.7</td>
<td>0.9</td>
<td></td>
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</table>
PBL counts was paralleled by an increase of large mononuclear cells, labelled with OKT4 or OKT8 or both, but only weakly by OKT3.

Modifications of lymph fluid cells occurred later than in blood, in parallel with a marked reduction of the lymphocyte counts (from $2.0 \times 10^6$ cells/ml to $0.7 \times 10^6$ cells/ml after the tenth day). The counts of OKT3$^+$, OKT4$^+$ and OKT8$^+$ lymphocytes decreased, but the BL2$^+$ lymphocytes were more increased than in blood (Table I). Interestingly, major modifications in the distribution of these four markers occurred closely to the tenth day. It involved a brief rise in OKT8$^+$ and BL2$^+$ cells which was followed by a drop of OKT3$^+$ and OKT4$^+$ cells. Unlike in blood, there was no appearance of a large cell subset. However, the comparison between the OKT3$^+$ cell counts and the sum of OKT4$^+$ and OKT8$^+$ cell counts suggests the presence of a double-labelled cell subset. The amplitude of these cellular modifications was lower in the patients from group B. One patient from group C received a kidney transplant four months after the end of the TDD, and his lymphocyte subsets were enumerated during this period. At the one hundredth day after TDD, the percentages of OKT3$^+$ and OKT8$^+$ lymphocytes remained about 10 per cent lower than the predrainage values. Moreover a profound reduction in OKT4$^+$ cells persisted (from 48.5% predrainage to 13.7% at the 100th day). This patient had no acute rejection episodes during the first year.

**Allograft function**

Irrespective of their groups A, B or C, the TDD patients were given the same immunosuppressive regimen at the time of the transplantation: azathioprine 2mg/kg/day adjusted to white blood cell counts; prednisone 1mg/kg/day progressively tapered; intravenous antilymphocyte globulins (ALG) 10 to 20mg/kg/day during the first four weeks.

Acute rejection episodes were treated by increasing steroid dosages to 15mg/kg/day (one day), then 4mg/kg/day (two days) followed by progressively reduced doses. ALG was administered again in some acute rejection episodes.

All the patients from this series received a cadaver kidney transplant, at a mean time of six weeks after the end of TDD (1–28 weeks). Statistical analysis (Student t-test) showed no significant difference between the three groups of patients with respect to age, sex, number of previous whole blood transfusions, anti-HLA alloimmunisation, and number of HLA compatibilities.

During the first five years after transplantation, the percentage of functioning grafts appears constantly better in group C than in group A (Figure 1). The differences in survival percentages are higher than 10 per cent, reaching 14.7 per cent at six months, but they are statistically significant ($p < 0.05$) only at one month.

Among the group B patients, there is a decrease of functioning grafts after the second year. Patients from group C experienced the lowest incidence of acute rejection episodes during the first year (Table II), resulting in a slightly lower cumulative steroid dosage than in the other groups. Patients from group B encountered more rejection episodes between the first and third month than those of group A.
Figure 1. Actuarial curves of kidney allograft survival in three groups of patients: unsuccessful TDD (group A), insufficient TDD (group B) and successful TDD (group C)
TABLE II. Actuarial percentages of patients experiencing first acute rejection episode during the first year

<table>
<thead>
<tr>
<th>Periods (months)</th>
<th>A</th>
<th>Groups B</th>
<th>C</th>
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</thead>
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<tr>
<td>1</td>
<td>66.7</td>
<td>61.5</td>
<td>43.6</td>
</tr>
<tr>
<td>3</td>
<td>81.0</td>
<td>92.3</td>
<td>64.1</td>
</tr>
<tr>
<td>6</td>
<td>85.7</td>
<td>100.0</td>
<td>71.8</td>
</tr>
<tr>
<td>12</td>
<td>85.7</td>
<td>100.0</td>
<td>74.4</td>
</tr>
</tbody>
</table>

Discussion

During the past 10 years, the few studies on TDD in human kidney transplantation suggested a beneficial effect of this immunological preparation [2–6]. However, there was some doubt on the long term improvement of allograft prognosis. The present results were obtained on a large number of patients selected for TDD according to the same criteria, and classified into three groups, that are similar with respect to all currently assessed immunological parameters. It appears unequivocally that a successful, even short, TDD reduces the number of acute rejection episodes within the first year after transplantation. Moreover, the subsequent increase of functioning grafts is maintained up to the fifth year.

The mechanisms of the immunosuppressive effect of a pre-transplant TDD still remain unclear. A major role cannot be attributed to the peripheral lymphopenia, since PBL counts return to the predrainage values after a few days. Previous studies described several modifications of T-cell function, including a decrease of delayed hypersensitivity skin reactions [7], and decreased proliferative responses to antigens or mitogens [9,10,14], despite a relatively preserved B/T lymphocyte ratios [14]. These modifications were already related to the replacement of the T-lymphocytes by a population of large cells bearing markers of early differentiation stages, as the Ia-like antigen [11]. The flow-cytometry monitoring of the T-cell subsets during TDD provides two major items of information. First, these results confirm, on other criteria, the appearance of less differentiated large cells, whose increasing number accounts for the decrease of OKT3+ and the increase of BL2+ lymphocytes. Secondly, there is a profound reduction of helper T-lymphocyte bearing OKT4 marker, of which the prolongation up to the third month after TDD is probably an important immunosuppressive factor. The unexpected enhancement of rejection episodes after an insufficient drainage could be accounted for by a ‘rebound’ phenomenon, although we do not have data supporting this hypothesis as yet.

In conclusion, pre-transplant TDD appears as a relatively safe and efficient immunosuppressive method. The improved allograft prognosis must be related to the modifications of T-cell subsets. Further studies are required to define the possible tolerising function of the TDD-induced immature T-cells, and to evaluate the subtle changes induced by short-term TDD among interacting subsets of lymphocytes.
References

1 Woodruff MFA, Anderson MF. *Nature* 1963; 200: 702
7 Machleder HI, Paulus H. *Surgery* 1978; 84: 157

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