LONG TERM OBSERVATION AFTER CULTURED HUMAN PARATHYROID CELLS ALLOTRANSPLANTATION WITHOUT IMMUNOSUPPRESSION


Department of General, Vascular and Transplant Surgery, Warsaw Medical University, Poland
Department of Pathology, Children’s Memorial Health Institute, Warsaw, Poland
Introduction

Several years ago we reported a new preparation strategy of the parathyroid gland endocrine tissue (Wozniewicz B, Migaj M, et al. Transplantation Proceedings 1996; 28: 3542)

A pure culture of active endocrine cells devoid of immunogenic antigens was obtained as a result of culturing and deep freezing of the cells before allotransplantation
The Strategy

The aim of parathyroid tissue preparation was to produce pure line endocrine cells for the treatment of patients with hypoparathyroidism, based on the facts that:

- parathyroid tissue constitutes only 50% of the mass of the gland
- class II HLA antigen bearing cells (endothelial cells, fibroblasts, lymphocytes, macrophages, etc.) and the remaining part of the parathyroid gland (the epithelial endocrine component) present only a weak expression of class I HLA
The Aim

The aim of the present study was to assess the long-term function of allotransplanted cultured parathyroid cells.
Indications for parathyroid allotransplantation

• Inadequate substitutional therapy for idiopathic or iatrogenic parathyroid insufficiency (ie. after thyroid surgery)

• Di George’s syndrome
Criteria of surgical hypoparathyroidism

• Typical symptoms of hypocalcemia noticed after the thyroid operation

• necessity of replacement therapy (vit. D and calcium supplementation)

• permanent hypocalcemia and hyperphosphatemia
Criteria of surgical hypoparathyroidism cont.

- serum parathyroid hormone (PTH) level below radioimmunoassay (RIA) sensitivity (PTH - normal range: 9-55 pg/mL, the lowest detection limit 1 pg/mL, IRMA - mat PTH - intact Byk-Santec Germany)
Materials and Methods

- Donor characteristics
- Recipient characteristics
- Culture technique
- Preparation for allotransplantation
- Allotransplantation technique
- Criteria for graft function
- Criteria for graft failure
- Posttransplantation monitoring
Donor characteristics

- 4 patients with secondary hyperparathyroidism associated with renal failure
- 3 patients with tertiary hyperparathyroidism after renal transplantation
Recipient characteristics

- 45 recipients
  - 43 female
  - 2 male

- recipient age
  - range (32 – 64 years)

- all patients were 2 – 14 years after thyroid surgery
Recipient characteristics cont.

- Dialy supplementation

  - 1-3µg of active vitamin D3 (Rocaltrol)
  - approx. 800-2400 mg elementary calcium
  - L-thyroxine 25-125 µg in 35 patients
Preparation for allotransplantation

• Only glands with diffuse hyperplasia (either fresh or stored) were selected for further culturing

• Parathyroid explants were screened to avoid incidental transplantation of carcinoma’s, adenoma’s and nodular hyperplasia
Culture technique

- 25 mL Falcon plastic flask
  - parathyroid cells suspension in Eagle’s medium supplemented with 10% FCS, 5mg/100mL Gentamicin, 0.02 mol/L L-Glutamine, NGF, ion calcium
Culture technique cont.

- Medium changed every 2-5 days
  - monitoring of PTH concentration in supernatant (only cultures with level PTH > 1000 pg/mL were used to subsequent investigation
  - removing nonproliferating parathyroid cells
  - selection of 100% pure population of PTH positive cells using panel antibodies
Culture technique cont.

• After six weeks
  ▪ a cultured monolayer of parathyroid cells reaching a density of 2 to 3 million cells per flask
• assessment of parathyroid cells viability using blue trypan
• unlimited time storage in liquid nitrogen
Culture procedure

- after morphological and functional assessment, the final parathyroid cell culture was cryopreserved before transplantation

- restorage procedures were started 24 hours before allotransplantation
Control of culture immunogenicity

- A panel of monoclonal mouse anti-human antibodies was used to investigate the glands for expression of immunogenic antigens.

- A transplantation trial of cultured parathyroid cells into a mouse abdominal cavity was performed as an additional assessment of the cells immunogenicity.
Allotransplantation technique

- A tiny incision of the skin and subcutaneous tissue of the nondominant forearm was performed to expose the fascia

- The cultured cells (approx. 20-30 million cells suspended in recipient serum) were meticulously injected exactly under the fascia
Criteria for graft function

- The PTH level in blood serum taken from the nongrafted forearm is higher than the pretransplant level.
- There is a PTH level gradient above 1.5 across the blood draining the grafted and ungrafted arms.
- Serum calcium levels rise to 2.0 mmol/L or more after transplantation without vit. D supplementation.
Criteria for graft failure

- The graft did not initially meet the criteria of function
- The serum calcium level returned to pregraft level if the patient did not receive supplementary therapy
- The serum PTH concentration substantially dropped or was below the sensitivity level for RIA
- The reinstatement of replacement therapy
### Number of allo-, re-allo and second re-allo transplantations

<table>
<thead>
<tr>
<th>Transplantation</th>
<th>Donors</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allo-</td>
<td>Donors without immunosuppression</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Donors with immunosuppression</td>
<td>22</td>
</tr>
<tr>
<td>Reallo-</td>
<td>Same donor</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Different donor</td>
<td>13</td>
</tr>
<tr>
<td>Second reallo-</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>62</strong></td>
</tr>
</tbody>
</table>
Histocompatibility complex assessment

- We discovered no relation between graft function and histocompatibility complex in the first 18 grafted patients
  - the presence of a common HLA antigen did not influence late transplantation results
- We did not find lymphocytotoxic antibodies in recipient blood serum
Posttransplantation monitoring

• In all recipients PTH-intact was measured in blood samples coming from the ipsilateral and contraleteral cubital vein in regard to the site of allografted cells.
• To exclude eventual ectopic parathyroid gland secretion, recipients with normal serum PTH levels over a period of 36 months had the same parameters measured after 10 minutes of pneumatic cuff placement on the ipsilateral arm.
Results

- Culture results
- Clinical results
Culture results

• Six weeks of parathyroid tissue culturing gives hormonally active but non-immunogenic endocrine cells
Immunopathologic and functional data on hyperplastic and normal parathyroid tissue prior and after culturing

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Tissue</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hyperplasia (n=10)</td>
<td>Normal (n=10)</td>
</tr>
<tr>
<td>HLA ABC</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HLA DR, DP, DQ</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>HLA DR antigen beta chain</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>HLA DR antigen alpha chain</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>PTH</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>T cell (CD3)</td>
<td>+++++</td>
<td>-</td>
</tr>
<tr>
<td>T-cell helper inducer (CD4)</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Tsuppressor/cytotoxic (CD8)</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>B cell (CD22)</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>Endothelial (CD31)</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Macrophage (CD68)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5B5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
PTH + HLA class I and II type antigen presentation before and after culturing
Human cultured parathyroid cells injected into a mouse peritoneal cavity confirmed the lack of immunogenicity (one year after xenotransplantation)
Clinical results

PTH level in grafted arm

- Gradient PTH = ____________________ $= 1,0-1,67$
  
  PTH level in non-grafted arm

- Values above 1,5 are an unquestionable evidence of transplant function
- The absence of PTH gradient does not rule out either the presence of functioning tissue or the capacity for PTH secretion
Clinical results

• Prior to transplantation, PTH in all cases was below the lowest detectable level

• In all patients after transplantation, PTH levels were higher in regard to before transplant surgery
Time of allograft function related to donor

- Immunosuppressed donor
- Non-immunosuppressed donor
Normal PTH levels - graft function or ectopic gland secretion?

• A decrease in PTH levels in a selected 5 patient group (with a functional graft above 36 months) was observed after pneumatic cuff placement.
Normal PTH levels - graft function or ectopic gland secretion?

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Grafted arm PTH (pg/ml)</th>
<th>Non-grafted arm PTH (pg/ml)</th>
<th>Non-grafted arm after tourniquet placement</th>
<th>Period of good graft function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.2</td>
<td>13.8</td>
<td>10.1</td>
<td>129</td>
</tr>
<tr>
<td>2</td>
<td>12.1</td>
<td>9.2</td>
<td>7.4</td>
<td>117</td>
</tr>
<tr>
<td>3</td>
<td>13.8</td>
<td>11.3</td>
<td>9.8</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td>18.8</td>
<td>16</td>
<td>12.7</td>
<td>46</td>
</tr>
<tr>
<td>5</td>
<td>23.2</td>
<td>19.8</td>
<td>14.3</td>
<td>36</td>
</tr>
</tbody>
</table>
Normal PTH levels - graft function or ectopic gland secretion?

- This indicates that the normal PTH levels depend solely on normal graft function and not on ectopic gland secretion.
Conclusions

- Allografted human cultured parathyroid cells show evident but transient hormonal activity.

- Among 25 of 45 recipients (55%), hormonal activity of the transplanted cells was observed for over 1 month.
Conclusions cont.

• The best donors of parathyroid cells were kidney transplant recipients on immunosuppression

• The mechanism of activity loss in parathyroid cells in the majority of recipients remains unknown, in some patients the mechanism of activity loss is other than immunological
Conclusions cont.

• The ease of use and good control makes cultured parathyroid cell allotransplantation a promising new therapeutic tool

• The experience gained in parathyroid cell grafting can be used in transplantation of other cells