Effect of Directly Ultraviolet-irradiated Allografts of Fetal Pancreas in Experimental Diabetic Dogs

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ABSTRACT. The proliferative responses of UV-irradiated islets from fetal pancreas decreased to 53.8±4.7% (mean ± SEM) compared to that of UV-irradiated islets by allogeneic mixed islet-cell lymphocyce culture. In 5 pancreactomyed dogs, UV irradiated fetal dog pancreas was transplanted either into omentum pouches or the spleen without immunosuppressive agents. The diabetic status (daily insulin requirement for hypoglycemia, decrease in body weight, urine glucose) improved in dogs after allo-transplantation. The survival time after total pancreatectomy was significantly longer in allografted dogs than those treated only with daily insulin injections. — Key words: diabetic canine, fetal pancreas transplantation, ultraviolet irradiation.


Transplantation of allogeneic islets isolated from adult pancreas or fetal pancreatic tissue can successfully reverse diabetes in rodents, but transplantation in larger animals is more complex [1]. Dogs and pigs have been most commonly used for various studies on transplantation as large animal models [2, 9, 10, 13]. Mullen et al. [13] have reported the reversal of hyperglycemia in diabetic pigs by transplantation of fetal pancreatic islets, but long-term survival of grafts required the continuous use of immunosuppressive agents. Kenyon et al. [9] have reported the prolongation of allograft survival by direct ultraviolet (UV) light irradiation of donor tissue combined with cyclosporine therapy. Graft survival has been prolonged by immunomodulation with UV light, either directly by irradiation of islets before transplantation, or indirectly by transfusion of UV-irradiated blood elements such as peripheral blood lymphocytes, plasma, or platelets [3, 4, 6, 11, 14, 15, 21]. The delayed type hypersensitivity response, mixed lymphocyte culture reaction and rejection of skin allografts were shown to be suppressed in dogs and miniature pigs after transfusion of UV-irradiated blood elements from the allogeneic donor [18, 20].

In rodents, passenger leukocytes and/or dendritic cells were reported to be inactivated by direct UV-irradiation of donor tissues or of donor peripheral blood lymphocytes to be used for transfusion [6, 7, 9]. Allograft survival was prolonged after UV-treatment, and tended to improve the diabetic status [6, 7, 9], but the effects of direct UV-irradiation of donor tissue in fetal pancreatic allografts have not been studied in dogs, although the dog is a very important large animal model for diabetes.

The purpose of this study is to examine the effect of transplantation of UV-irradiated allografts of fetal pancreas on experimental diabetic dogs.

MATERIALS AND METHODS

Animals: Ten mongrel dogs, 6–24 months old and 8–14 kg, were used as recipients. The dogs underwent total pancreatectomy to induce stable diabetes. The dogs were divided into two groups as follows: allo-transplantation (n=5, group 1) and no transplantation (n=5, group 2).

Treatments of diabetes: These were carried out as described previously [19]. Briefly, plasma glucose levels were maintained at 250 mg/dl by administering neutral protamine hagedron (Shimizu Pharmaceutical Co., Ltd., Japan) and regular insulin (Shimizu Pharmaceutical Co., Ltd., Japan) for the experimental period. The dogs received a daily standard meal consisting of 50–70 kcal/kg of a commercial dog food made of beef, and were given 10 mg/kg of cimetidine (Fujisawa Pharmaceutical Co., Ltd., Japan) and 220 mg/head of pancreatic exocrine enzyme (Maruhou Co., Ltd., Japan) twice a day. They were given free access to water and prevented from ingesting fecal material.

Preparation of fetal pancreatic grafts: Fetal pancreatic tissues, 45 to 60 days of fetal age, were obtained from dogs by terminating pregnancies. The tissues were cultured for 24 hr in medium (see below) at 37°C in a 5% CO2-humidified atmosphere, after either cutting the tissue into fragments or collagenase digestion (5 mg/ml Sigma Type 5, U.S.A.). Three to five pieces of fetal pancreatic tissue, with or without collagenase treatment, were transplanted into omentum pouches or the spleen after direct UV-irradiation, without the use of any immunosuppressive agents.

The culture medium consisted of RPMI1640 (05911 Nissui Pharmaceutical Co., Ltd., Japan) supplemented with 8% inactivated fetal calf serum (FCS, Bioproducts, U.S.A.), pyruvic acid (1.2 mM), L-glutamine (3.4 mM), 2-mercaptopethanol (5×10-5 M), penicillin (0.1 mg/ml), streptomycin (0.1 mg/ml), gentamicin (0.05 mg/ml), fungisone (0.25 mg/ml), HEPES buffer (25 mM), and sodium bicarbonate (24 mM).

Ultraviolet light irradiation: The grafts were suspended in 5 ml aliquots of RPMI1640 containing 1% FCS, placed at 10 cm from the UV light source (model UVM-57, 302 nm, 520 W/cm²–15 cm, UVP Inc., San. Gabriel, CA,
U.S.A.), and irradiated for 10 min on a reciprocating shaker (model SR-1, Taiyo Science Co., Ltd., Japan).

Mixed islet-cell lymphocyte culture: The recipient peripheral blood lymphocytes as responder cells were obtained by gradient centrifugation in a Ficoll-Paque centrifuge (specific gravity: 1.077, 17-0840-03 Pharmacia, U.S.A.), washed twice in phosphate buffered saline, and once in RPMI1640 containing 1% FCS. After washing, the cells were resuspended in RPMI1640 containing 8% FCS. Stimulator cells of fetal pancreas after collagenase digestion and responder cells were treated with mitomycin C (0.04 mg/ml, Kyowa Hakko Kogyo Co., Ltd., Japan). The cells were cultured in 96-well U-bottomed microtiter plates (3910, Falcon, U.S.A.), with 5×10⁵ stimulator cells and 5×10⁶ responder cells per well (0.2 ml/well). The cultures were maintained at 37°C in a 5% CO₂ humidified atmosphere for 5 days. Four hours before the end of the culture, 0.01 ml/MTT [3-(4,5-dimethyl thiazol-1-2-ly)-2,5-diphenyl] tetrazolium bromide, 5 mg/ml, Wako Pure Chemical Ind., Ltd., Japan] was added to each well. After 4 hr, the medium of each well was removed, and then 0.1 ml dimethyl sulfoxide was added to each well to dissolve the formazan crystals. Optical density was measured with a microplate reader (MTP-32, measurement wavelength 550 nm, reference wavelength 630 nm, Corona Electric, Japan). Proliferation of the mixed islet-cell lymphocyte cultures was calculated as stimulation index = experimental density to the control density. The control for mixed islet-cell lymphocyte cultures was the proliferation of an analogous culture consisting of untreated responder cells and stimulator cells from the same animal, treated with mitomycin C. The percent stimulation index was calculated as the ratio of the stimulation index (UV-irradiation) to the stimulation index (no-irradiation) of the mixed islet-cell lymphocyte culture × 100.

Observation of diabetic status: Plasma glucose levels, daily insulin requirements, urine glucose levels, serum insulin concentrations and body weight were measured in the dogs with induced diabetes to monitor the diabetic status. Plasma glucose levels were measured by a dry chemistry method (Fuji Medical System Co., Ltd., Japan). Serum insulin concentrations were measured by an enzyme immunoassay (Wako Pure Chemical Ind., Ltd., Japan).

Statistical analysis: Results are expressed as the mean ± SEM (standard error of mean). Statistical significance was analyzed by paired or unpaired Student's t test. Differences with p<0.05 were considered statistically significant.

RESULTS

Effect of direct UV-irradiation on mixed islet-cell lymphocyte culture: The percent stimulation index with UV-irradiation significantly decreased to 53.8±4.7% of that found with non-UV-irradiated tissue (Table 1). After direct UV-irradiation of the stimulator cells, the very high (80-90) viability of the islet-cells was identified by their inability to exclude trypan blue.

Effect of allografting with UV-irradiated fetal pancreas: All the dogs became hyperglycemic within 24 hr after total pancreatectomy. Fasting plasma glucose levels were 404.0±9.3 mg/dl. Survival in the two groups after total pancreatectomy is shown in Table 2. In group 1 (allo-transplantation), the survival period was 168.8±34.1 days, which was significantly longer than that of group 2 (no transplantation), 24.8±6.5 days. In addition, two dogs (DM-No. 2 and 3) in group 1 survived more than 180 days. In particular, DM-No. 3 remained in good condition for

<table>
<thead>
<tr>
<th>Group (treatment)</th>
<th>n</th>
<th>Days of survival after total pancreatectomy (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Allo-Tx&lt;sup&gt;0&lt;/sup&gt;)</td>
<td>5</td>
<td>69, &gt;128, 164, 183, &gt;300 (168±34.1)&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 (No-Tx)</td>
<td>5</td>
<td>8, 12, 27, 28, 49 (24±6.5)</td>
</tr>
</tbody>
</table>

a) Tx: Transplantation.  
b) Significant difference, p<0.01 vs. no-Tx.

d) Serum insulin concentrations.

e) Pre-transplantation/Post-transplantation levels.  
Indicated by mean levels for experimental periods.

Table 1. Effect of UV-irradiation on mixed islet-cell lymphocyte culture (MILC)

<table>
<thead>
<tr>
<th>% Change in MILC stimulation index&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-UV</td>
</tr>
<tr>
<td>UV</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>53.8±4.7&lt;sup&gt;b&lt;/sup&gt; (n=5)</td>
</tr>
</tbody>
</table>

<sup>a</sup> SI = density of allogenic MILC  
<sup>b</sup> density of autologous MLR

Table 3. Individual diabetic status data of all dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>DM-No.</th>
<th>DRI&lt;sup&gt;d&lt;/sup&gt; (mU/l)</th>
<th>UGL&lt;sup&gt;e&lt;/sup&gt; (g/kg/day)</th>
<th>PGL&lt;sup&gt;e&lt;/sup&gt; (mg/dl)</th>
<th>SIC&lt;sup&gt;e&lt;/sup&gt; (µU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>46/389&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10/14</td>
<td>207/237</td>
<td>N.D./2.9</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>85/648</td>
<td>8/8</td>
<td>343/224</td>
<td>6.2/7</td>
</tr>
<tr>
<td>Allo-Tx</td>
<td>3</td>
<td>86/326</td>
<td>21/0</td>
<td>301/256</td>
<td>18.5/18.4</td>
</tr>
<tr>
<td>4</td>
<td>75/66</td>
<td>3/0</td>
<td>385/267</td>
<td>5.215/5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>77/616</td>
<td>26.5</td>
<td>278/232</td>
<td>4.9/23.3</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Daily insulin requirements.  
<sup>b</sup> Urine glucose levels.  
<sup>c</sup> Plasma glucose levels.  
<sup>d</sup> Serum insulin concentrations.  
<sup>e</sup> Pre-transplantation/Post-transplantation levels.

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more than 300 days. The diabetic state of the allografted dogs is shown in Table 3. The diabetic parameters of DM-No. 3 showed the greatest improvement, as indicated in Fig. 1. Daily insulin requirements to control hyperglycemia significantly reduced from 740.3±51.6 mU/kg/day to 580.1±19.9 mU/kg/day after transplantation, as shown in Fig. 2. Of particular interest, the mean insulin dose required to control hyperglycemia in DM-No. 3 was high (863 mU/kg/day) before transplantation, but after transplantation, decreased gradually to 529 mU/kg/day at 15 weeks (Fig. 1) and remained at this level for more than 300 days. The doses for the other dogs also decreased after transplantation. Although transplantation treatment did not achieve a gain in mean body weight in the transplantation group, the body weight of DM-No. 3 increased after transplantation. Before transplantation, the body weight of DM-No. 3 decreased from 12.9 kg to 11.6 kg in spite of the administration of insulin, but it increased to that level before the induction of diabetes 21 weeks after transplantation (Fig. 1), and continued to increase to 13.5 kg at 42 weeks. Urine glucose levels showed a tendency to improve from 11.0±5.9 g/kg/day to 9.7±2.4 g/kg/day after transplantation (Fig. 2). DM-No. 1 showed a decrease in urine glucose levels after transplantation (Table 3). However, the levels in the other dogs did not significantly reduce compared with the pre-transplantation levels. The plasma glucose levels increased to 311.1±10.4 mg/dl after total pancreatectomy, but fell to 243.1±4.6 mg/dl after transplantation (Fig. 2). Plasma glucose levels of DM-No. 3 decreased to 213 mg/dl by 3 weeks after transplantation, and daily insulin requirements decreased compared to pre-transplantation levels (Fig. 1). Weekly monitoring of serum insulin concentrations showed an increase from 6.5±1.3 to 11.2±2.1 μU/ml after transplantation (Fig. 2). The level in DM-No. 3 also increased (Fig. 1).

**DISCUSSION**

In rodents, cultured islet and purified islet allografts resulted in prolongation of recipient survival [5, 8], but the few passenger leukocytes that escape inactivation can theoretically trigger the rejection process. Ultraviolet (UV)-irradiation of rat dendritic cells completely ablished their allostimulatory capacity in mixed lymphocyte culture, and prolonged allograft survival without the use of immunosuppressive agents [7]. In our investigation in dogs, suppression of the response of mixed islet-cell lymphocyte culture was observed in UV-irradiated islets and this result indicates that it is possible to attenuate the immunogenicity of canine fetal pancreatic tissue without killing the islet cells.

An advantage of the fetal pancreas is the lower proportion of exocrine tissue than in adult pancreas. In addition, collagenase digestion and culture allow the growth of endocrine tissue whereas the exocrine elements

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* This indicates that the difference, p<0.05 vs. before transplantation.
disintegrate [12]. In the present study, transplantation did not result in complete reversal of the diabetic state, but the effect of allograft direct UV-irradiation was observed. Plasma glucose and urine glucose levels and also the requirement of daily insulin decreased. Particularly in DM-No. 3, an obvious improvement in the diabetic state and a gain in body weight was observed over a long period after transplantation, without the use of any immunosuppressive agents. The present study also demonstrated the beneficial effect of direct UV-irradiation on allotransplantation of fetal pancreas, because the acute rejection of non UV-irradiated pancreatic allografts has been described in many experiments in rodents [6, 7, 16] and also in the dog [9]. Histological examination and immunocytochemical staining of fetal pancreatic grafts did not detect insulin producing cells, but the use of direct UV-irradiation was able to reduce canine fetal pancreatic immunogenicity. The change in the characteristics of fetal pig pancreas associated with the fetal growth have been reported [17]. In this investigation, pancreatic tissue was taken from fetuses of gestational age 45 to 60 days, but the optimal age for the cure of diabetes has not yet been defined. The amount of donor pancreas and the location of transplantation are also under debate.

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REFERENCES