Allogene Pancreas Transplantation In Streptozotocin Diabetic Inbred Rat Strains: Impact Of Presensitization On The Pattern Of Rejection
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Citation

Abstract
Aim of the study: In a family of congenic and recombinat rat strains the pattern of rejection of vascularized pancreas allografts in presensitized recipients was studied in order to make a special time frame of rejection available. Donor and recipient differed either in their entire MHC or in class I or class II MHC antigenes.

Material and Methods: In streptozotocin diabetic (55 mg/kg bone-weight) recipients an intraabdominal heterotopic pancreas whole organ transplantation was performed. The exocrine secretion of the pancreas was suppressed using a ligation of the duct. Rejection was defined as recurrence of diabetic hyperglycemia exceeding 14 mmol/l and subsequent confirmed bei histologic examination.
The pancreas recipients were presensitized by repetitive donor-specific skin-transplantation. Full skin transplantation was performed 12, 8 and 4 weeks prior to pancreas transplantation.

Results: The results show an accelerated rejection in all instances: In the entire MHC incompatibility the median rejection time in native recipients was defined with 12,5 days, in presensitized recipients with 8 days. In class I MHC incompatibility native recipients rejected the allografts after median 16,5 days, presensitized recipients after median 9 days. Concerning class II MHC incompatibility rejection in native recipients set in in native recipients after median 16 days, in presensitized recipients after median 7 days. A hyperacute rejection however could not be observed.

Discussion: The results show an accelerated pattern of rejection in all instances caused by donor MHC antigenes. A hyperacute rejection however could not be observed. The effect is slightly pronounced in class II MHC incompatibility. Acceleration of rejection may be reasoned in a stimulation of the humoral as well as the cellular pathway of immune response and requires in clinical routine a special maintenance. The underlying mechanism concerning the different MHC incompatibilities however remains speculative.

INTRODUCTION
Diabetes in younger individuals, commonly known as insulin-dependent-diabetes-mellitus, is still a serious problem (1). Basic strategy of drug-therapy consists of subcutaneous application of the different forms of insulin (2). Remembering a persistent well adjustment of the blood glucose level however Diabetes causes long-term complications, such as small vessel disease (3), diabetic nephropathy (4) or diabetic neuropathy (5). Because of this pancreas transplantation can represent a successful alternative to conventional drug therapy (6).

The technical aspects of pancreas transplantation do not represent the major problem nowadays (7), however immunologic problems i. e. allograft rejection are actually present (8). The whole pancreas mainly consists (95 %) of exocrine tissue which is not relevant for the blood glucose level, so that isolated transplanted islets of Langerhans would be sufficient to manage the glucose metabolism. But even endocrine tissue is overcome by alloimmunreactions, however with a milder pattern of rejection (9, 10). The main problem represents the measure of islets that is required for only one recipient in allograft transplantation.

Incompatibility of tissue is known as one of the basic problems in organ transplantation. The most important transplantation antigenes are determined in the major
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histocompatibility complex (MHC). Class I and class II antigens with a different pattern of organ distribution are known (11).

Aspired is the highest possible degree of tissue compatibility in order to avoid organ failure that is caused by rejection.

Concerning pancreas transplantation clinical data (12) have been relatively rare but already increased in the last decade and a presensitized patient represents a special problem case: Some patients have already undergone organ transplantation and by this they may are disposed with preformed antibodies which are able to complicate subsequent pancreas transplantation. Moreover other sources of presensitization such as blood transfusion are pregnancy can complicate subsequent organ transplantation.

In other organs, e.g. for the kidney, it is known that presensitization can trigger a hyperacute rejection. Concerning pancreas, however, exact data are relatively rare. So we performed this experimental study in order to construct a definite time frame of rejection in presensitized recipients under special consideration of the dependency on the underlying genetic incompatibility. The pattern of rejection throughout the entire MHC as well as class I and class II MHC incompatibility was studied and compared to unmodified pancreas whole organ transplantation.

MATERIAL AND METHODS

For the transplantation experiments 3-4 weeks old male rats with a body weight between 180 and 300 g were used. The carriage was performed under standardized conditions and free access to water and food.

In all mammal species inclusive men the most important transplantation antigenes are coded by the major histocompatibility complex (MHC). The MHC consists of a few narrow linked gene-loci and shows a homologous pattern between rat, mouse and man (13, 14, 15). The MHC of the rat is called RT and concerning man HLA. In rat class I antigenes are coded by the gene-regions RT1.A and RT1.C, class II antigenes are coded by the gene-region RT1.B. Furthermore histocompatibility antigenes are known which are coded outside from the MHC and represent so called non-MHC antigenes. The following table gives an overview concerning the used strain combinations:

<table>
<thead>
<tr>
<th>Incompatibility</th>
<th>donor-strain</th>
<th>RT1</th>
<th>recipient-strain</th>
<th>RT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-MHC</td>
<td>AS</td>
<td>111</td>
<td>LEW</td>
<td>111</td>
</tr>
<tr>
<td>MHC</td>
<td>LEW1A</td>
<td>aaa</td>
<td>LEW1U</td>
<td>uuu</td>
</tr>
<tr>
<td></td>
<td>LEW1A</td>
<td>aaa</td>
<td>LEW1R6</td>
<td>uaa</td>
</tr>
<tr>
<td></td>
<td>LEW1R6</td>
<td>aaa</td>
<td>LEW1R4</td>
<td>uaa</td>
</tr>
<tr>
<td></td>
<td>LEW1A</td>
<td>aaa</td>
<td>LEW1R3</td>
<td>uaa</td>
</tr>
</tbody>
</table>

The experimental operations were performed in a microsurgical laboratory under clean but not sterile conditions. As optical aid an operation microscope (OPMI I, Company Zeiss) was employed.

ANESTHESIA

Donor-operations were performed using intramuscular injection of 0.5 ml (80-120 mg/kg bw) Rompun® 2% (Xylocainhydrochlorid, Bayer company) and 0.5 ml Ketanest® (80-120 mg/kg bw; Ketamin Parke-Davis company). For the recipient operation intraperitoneal ketamin anaesthesia (80-120 mg/kg bw) was used.

SKIN TRANSPLANTATION

Only full-skin allografts were used which -after skin excision in the recipient- could be transplanted without sutures but with help of a self stickening tape binding. After removing of the tape a daily inspection concerning the degree of rejection was performed. As day of rejection a complete necrosis of the allograft was classified. Skin transplantation was performed donor-specific repetitively 12, 8 and 4 weeks prior to pancreas transplantation.

INDUCTION OF A CHEMICAL DIABETES MELLITUS USING STREPTOZOTOCIN

A chemical Diabetes mellitus was induced using streptozotocin (2-Deoxy-2-methylnitrosamin-carbonyl-amin-D-Glucopyranose, Sigma chemicals company, USA) in the presensitized recipients 2 days before pancreas whole organ transplantation. Streptozotocin was soluted in Phosphoric buffered solution at pH 4.5 and applied in a dose of 55 mg/kg bw. Pancreas transplantation was only applied to diabetic animals with blood glucose levels > 18 mmol/l and clinical manifestations of diabetes mellitus with polyurie and polydypsie.

VASCULARIZED PANCREAS TRANSPLANTATION

The microsurgical technique is based on the work of Lee and
Nowaza (16, 17). Further development was performed by Klempnauer (18) in the own laboratory. Aim of the donor operation was an entire pancreatectomy in order to transplant as many islets of Langerhans as possible. The exocrine secretion was suppressed by closing the duct. Isolated pancreas was perfused in situ with 4atsu physiologic sodium chloride solution and subsequently preserved in iced solution of sodium chloride for no longer than 60 minutes.

In the streptozotocin diabetic rats a heterotopic intraabdominal implantation with systemic venous drainage was performed.

CONTROL OF ALLOGRAFT FUNCTION

General health condition, body weight and blood glucose level were defined during the first 100 postoperative days every day, and during the first 24 postoperative hours every 6 hours. The recurrence of Diabetes mellitus with blood glucose levels > 14 mmol/l occurring at 2 consecutive days was defined as rejection, so that the animals had to be sacrificed and underwent autopsy. Rejection was confirmed by histologic examination.

DETECTION OF ANTIBODIES USING THE CYTOTOXIC TWO-PHASES-TEST

One day prior to the second respectively third skin transplantation serum of the recipient animals already had been asserved employing a retroorbital venous punction. The test method itself represents a modification established by Gorer and O’Gorman (36): Lymphnodes of allogenetic to the donor strains identical but native animals were acquired and their cell-activity was controlled with trypan-blue (0,16%). In a following step 50 l of the freshly suspended lymphnode cells were mixed up with 50 l of antiserum solution from the recipient animals and incubated in a 37 celsius warm bath of water for 30 min.. Following a centrifugation was performed and the overstood was repudiated. After addition of complement the sediment was resuspended and once more incubated in a 37 celsius warm bath of water for 60 min., respectively concerning RT 1.C incompatibility 180 min. Then 20 l of trypan-blue was added and the percental part of lysed cells was determined.

HISTOLOGY

The explanted tissue was fixed in Bouin’s solution and embedded for light microscopy investigation in paraffine. Afterwards paraffine sections with a thickness of 5-7 m were prepared in order to perform a micoscopic examination in hematoxyline-eosine. Moreover such probes in which rests of islet cells could be observed underwent immunhistochemical couloring and examination concerning the established islet hormons.

STATISTICAL ANALYSIS

For descriptive statistics the median value was provided, for estimation of statistical significance the p-value of the Mann-Whitney-U-Test was employed. A p-value < 0,05 was considered to be statistically significant.

RESULTS

SKIN TRANSPLANTATION

Altogether 141 skin transplantations were performed in 50 animals. Remembering the described technique all transplantaions ran uneventful. Cases of death caused by anesthesia will not be considered in the following course. The following table describes the rejection times after skin transplantation:

Figure 2

<table>
<thead>
<tr>
<th>Incompatibility</th>
<th>Transplantation</th>
<th>Allograft Survival (days)</th>
<th>Mann-Whitney-U-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC 1st (unmodified)</td>
<td>6</td>
<td>7</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>2nd</td>
<td>6</td>
<td>5</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>3rd</td>
<td>6</td>
<td>7</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>RT1A 1st (unmodified)</td>
<td>8</td>
<td>9</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>2nd</td>
<td>8</td>
<td>7</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>3rd</td>
<td>8</td>
<td>7</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>RT1B 1st (unmodified)</td>
<td>13</td>
<td>7</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>2nd</td>
<td>13</td>
<td>7</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>3rd</td>
<td>13</td>
<td>7</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Non-MHC 1st (unmodified)</td>
<td>10</td>
<td>8</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>2nd</td>
<td>10</td>
<td>8</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>3rd</td>
<td>10</td>
<td>8</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

As expected the strongest acceleration of rejection takes place in the entire MHC incompatibility. After all a statistically significant acceleration of rejection can be mentioned in all cases, except for the second set transplantation in RT1.A incompatibility.

PANCREAS TRANSPLANTATION

Altogether 47 pancreas-whole-organ-transplantations were performed in 47 presensitized recipients. Three animals already had died in concern of anesthesia in skin transplantation and will not be considered in the following course.

In one case an insufficiency of the anastomosis led to the dead of the animal. It will not be considered int the following course, either.
Two animals of the non-MHC group and one of the MHC group died without hyperglycemia. Subsequent histology however showed severe signs of rejection. The timepoint of rejection was set equal to the point of death in these cases.

The following table gives an overview concerning the rejection times in presenstized pancreas recipients compared to native (not presenstized) recipients:

**Figure 3**

Table 3: This table gives a comparison distinguishing presenistized and native pancreas allograft recipients.

<table>
<thead>
<tr>
<th>incompatibility</th>
<th>presenstization</th>
<th>survival (days), median</th>
<th>Mann-Whitney-U-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC</td>
<td>Yes</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>12.5</td>
<td><em>p&lt;0.05</em></td>
</tr>
<tr>
<td>RT1.A</td>
<td>Yes</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>16.5</td>
<td><em>p&lt;0.05</em></td>
</tr>
<tr>
<td>RT1.B</td>
<td>Yes</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>16</td>
<td><em>p&lt;0.05</em></td>
</tr>
<tr>
<td>RT1.C</td>
<td>Yes</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>8</td>
<td><em>p&lt;0.05</em></td>
</tr>
</tbody>
</table>

Obvious from statistical analysis is, that an acceleration of rejection has taken place in any case. Even the fast median rejection time in the entire MHC group could be significantly accelerated to eight days of allograft survival. Besides that, the acceleration of rejection is slightly pronounced in class II incompatibility.

**GENERAL PHYSICAL CONDITION OF THE ANIMALS**

Streptozotocin in a dose of 55 mg/kg bw induced in all animals metabolic signs of Diabetes mellitus. In less than a few days all animals’ health conditions worsened and they lost their motoric activity, got a sheveled coat and lessened their body weight.

However, somedays following pancreas transplantation all physical signs of diabetes mellitus vanished and the animals got back their motoric activity.

At recurrence of diabetes all animals showed once more all diabetic signs with hyperglycemia, polydipsie and polyphagie. Once more they lessened their weight and general conditions got worse again.

**RESULTS OF BLOOD GLUCOSE DETERMINATION**

MHC incompatibility: After median 8 days (longest period 11 days) all animals, except for one, developed hyperglycemia with a blood glucose level exceeding 14 mmol/l. The exception however showed severe signs of rejection in the subsequent histology. As day of rejection the day of death was provided.

RT1.A incompatibility: The recurrence of diabetes occured after median 9 days, the longest period however was measured within 11 days.

RT1.B incompatibility: The diabetic hyperglycemia concerning this disparity set in after median 7 days, the longest period ran up to 13 days. Sporadically prognostics of a graft-versus host reaction with erytheme, alopecia and hyperceratosis in the area of the legs and ears could be observed.

RT1.C incompatibility: After median 30 days the diabetic signs occured back. Remarkable however remain three animals which showed prolonged organ function up to 100 days, i. e. until the finish of the observation span.

Non-MHC incompatibility: Organ failure set in after median 8 days, the longest period took 12 days. Once more in two cases the blood glucose level did not exceed the demanded 14 mmol/l. The autopsy again demonstrated however again macroscopic as well as histologic changes of rejection.

**RESULTS OF THE CYTOTOXIC TWO-PHASES-TEST**

In the following course the development of cytotoxic antibodies in each case of genetic incompatibility always before the second set of skin transplantation is exemplarily exposed.
Figure 4
Graphic 1 shows the production of cytotoxic antibodies concerning the entire MHC incompatibility before the second skin transplantation. In most solutions a severe lysis of cells could be observed. It is especially remarkable that the degree of lysis before the third set of skin transplantation has increased up to 80-100%, these results however are not demonstrated in a graphic.

Figure 5
Graphic 2 shows the production of cytotoxic antibodies concerning the isolated RT1.A incompatibility before the second set of skin transplantation. In only three instances the lysis exceeds values bigger than 20% (background toxicity of lymph-node cells). Before the third skin transplantation the degree of lysis leads up to 60-80% concerning the mentioned instances.

Figure 6
Graphic 3 demonstrates the antibody production concerning isolated RT1.B incompatibility before the second skin transplantation. Only in some cases the background toxicity of lymph-node cells is exceeded significantly. This, however increases before the third set of skin transplantation up to 60% of lysis in these recipient animals.

Figure 7
Graphic 4 shows the production of cytotoxic antibodies before the second skin transplantation concerning isolated RT1.C incompatibility. In no case a significant antibody production has set in. However before the third set of skin transplantation the observed lysis increases up to 40-70% in altogether four cases.
Figure 8

Graphic 5 demonstrates the production of cytotoxic antibodies before the second skin transplantation in the non-MHC incompatibility. In no case the background toxicity is overstepped significantly, this pattern doesn’t change at all before the third set of skin transplantation, even independent on the degree of the antiserum-solution.

HISTOLOGY

All examined allografts showed severe signs of rejection with infiltration of tight sticky tape and inoculation of immune cells such as granulocytes, lymphocytes or macrophages. In the undergoing endocrine tissue special islet-hormones couldn’t be observed anymore.

DISCUSSION

The indication for clinical pancreas transplantation is commonly combined with the indication for simultaneous kidney transplantation or already performed kidney transplantation (19).

Immunologic investigations have already shown that donor-specific kidney transplantation develops a benefit effect on subsequent transplanted pancreas whole organ grafts (22, 23). Only in a minor number of cases pancreas transplantation is performed without presence of terminal kidney insufficiency, because of life threatening hypoglycemia caused by conventional insulin therapy (19).

Clinical experience with pancreas whole organ transplantation has increased in the last decade (24). The main surgical problems, such as portal venous or systemic venous drainage could be solved in basic (25), however the immunologic response and the consequently following immunosuppressive drug therapy represent the major and remaining problems nowadays.

In general the destiny of allograft survival is determined by the degree of histocompatibility (25). The mentioned MHC antigens induce alloimmune responses in different intensities. The MHC of rat, mouse and man is homologous and consists of a various number of narrow linked gene loci (13, 14, 15). Because of function, distribution pattern and molecular structure class I and class II antigens can be differed (25, 26). Concerning the MHC expression principally three compartments are known: Organ-specific parenchyme, interstitial tissue and the vessel system (27). The MHC expression in the vessel system is in all organs identical (28): Class I antibodies respond to the endothelium of arteries, veins and capillaries, whereas class II antigens are not expressed in a normal standard there (29). In case of acute rejection however, a fast induction of expression of class II antigens on all vessel endothelium can be observed (30). The findings from our data that the acceleration of rejection is slightly pronounced in class II incompatibility may be explained by an overshooting induction of gene expression in case of donor-specific presensitization. The definite underlying immunological mechanism however remains speculative.

Besides that organspecific differences which mainly concern the organ parenchyme of the MHC expression are known: In a normal standard class I antigens are expressed on ductal epithelium cells and the islets of Langerhans, but not on acinus cells (31). Class II antigens are normally not expressed on ductal epithelium cells and acinus cells, however their expression is induced during acute rejection. A remarkable exception from this represent the islets of Langerhans which are free from class II antigens in a normal standard as well as during acute rejection (32).

Coming up to the clinical expectancy in such strongly presensitized recipients a hyperacute rejection should have taken place as it is known from kidney transplantation (33). This phenomenon however could not be observed in our data. In a special view the pancreas as whole organ may be overcome with high risk by pancreatitis due to explantation and cold ischemic time (28). Under these circumstances especially in presensitized recipients a hyperacute rejection seems to be more probable. After all the time frame of the (suspected) rejection times has to be defined once more: The expected hyperacute rejection has not set in, but an accelerated rejection throughout all instances slightly favorable in class II incompatibility.

The chosen model of repetitive skin transplantation is well known for antibody production and obviously from the cytotoxic two-phases-test a significant antibody production
has set in even before the second set of skin transplantation concerning the most major loci of the MHC. This effect is even more emphasized before the third set of skin transplantation. But nevertheless no hyperacute rejection has taken place.

CONCLUSION

Summing up all the results we may draw the conclusion that pancreas whole organ transplantation still remains a serious problem in clinical routine. The experimentally defined time frame however is an accelerated and no hyperacute rejection though significant levels of antibodies are present. This seems to be an interesting aspect under consideration of the special problem concerning the exocrine tissue of the pancreas with the danger of pancreatitis. The acceleration of rejection is slightly pronounced in class II of MHC incompatibility. From this can be postulated that those mismatched recipients are in the highest risk of organ rejection and probably should be led to specific therapy - e. g. plasmapheresis - (s) or excluded from transplantation.

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