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ORIGINAL ARTICLE

Insulin and islet autoantibodies after pancreas transplantation

Christoph D. Dieterle,[1] Franz-Xaver Hierl,[1] Bodo Gutt,[1] Helmut Arbogast,[2] Georg R. Meier,[1] Martin Veitenhansl,[1] Johannes N. Hoffmann[2] and Rüdiger Landgraf[1]

1 Diabetes Center, Medizinische Klinik, University of Munich, Munich, Germany
2 Transplant Surgery, Department of Surgery, Klinikum Grosshadern, University of Munich, Munich, Germany

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Correspondence
Dr Christoph Dieterle, Diabetes Center, Medizinische Klinik, Ziemssenstr.1, 80336 München, Germany. Tel.: 0049-89-5160-2363; fax: 0049-89-5160-2968; e-mail: christoph.dieterle@med.uni-muenchen.de

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Summary

Autoimmune recurrence and subsequent diabetes after pancreas transplantation has been described. In this cross-sectional study 91 type 1 diabetic patients were examined after successful pancreas/kidney transplantation (SPK). We studied the prevalence of autoantibodies to insulin (IAA), glutamate decarboxylase (GAD) and tyrosine phosphatase (IA-2) as well as parameters of pancreas graft function. Graft recipients were grouped according to immunoreactivity: group 1: no immunoreactivity; group 2: immunoreactivity to one antigen; group 3: immunoreactivity to two or three antigens. Twenty-five percent of graft recipients displayed no immunoreactivity, 39% displayed positivity for one antigen and 36% were positive for two or three antigens. There were no significant differences concerning fasting glucose, $HbA1_c$, glucose tolerance and renal function between the groups. Patients with cyclosporine ($n = 42$) as first-line immunosuppression displayed more often immunoreactivity to IA-2 and IAA than patients treated with tacrolimus ($n = 49$) (31% vs. 14%, $P = 0.04$; 67% vs. 47%, $P = 0.04$). In addition methylprednisolone therapy was related to less immunoreactivity to IA-2. Immunological markers for type 1 diabetes can be determined in the majority of pancreas graft recipients despite adequate immunosuppression. However, immunoreactivity was not associated with impaired graft function. Patients with cyclosporine for immunosuppression and withdrawal of glucocorticoids therapy were more often immunoreactive to IAA and IA-2.

Introduction

Type 1 diabetes mellitus results from immune-mediated selective destruction of pancreatic islets. In diabetic patients the detection of insulin or islet autoantibodies confirms the diagnosis of type 1 diabetes. At the time of disease onset, circulating autoantibodies are detectable in about 90% of patients [1]. The detection of autoantibodies precedes the clinical onset of the disease [2]. During the course of disease, antibody levels decrease or disappear, in other patients auto-imunoreactivity persists, however [3,4].

Insulin autoantibodies (IAA) are present before administration of exogenous insulin [5,6]. A distinction to insulin antibodies, caused by exogenous insulin, is not possible, however. IAA are present in about 60% of newly diagnosed type 1 diabetic patients, and more often in children [7,8]. Antibodies to glutamate decarboxylase (GAD) are detectable in 60% of patients at diagnosis of type 1 diabetes [9]. Antibodies to tyrosine phosphatase (IA-2) are highly specific for type 1 diabetes and detectable in 60% of type 1 diabetic patients [8].

Simultaneous pancreas–kidney (SPK) transplantation is the standard therapeutic option for uremic type 1 diabetic

patients [10–12]. Successful pancreas transplantation in type 1 diabetic patients can sustain insulin independence for indefinite periods while glucose and $HbA1_c$ values are normalized [10].

Pancreas transplantation represents a second exposure of the immune system to beta-cell autoantigens much later after the destruction of innate islets. Reappearance of islet autoantibodies after pancreas transplantation was reported in several studies. The meaning and consequences of recurrence of autoantibodies after allogenic transplantation concerning graft function and graft survival is still discussed controversially [13–15]. Recurrence of disease with hyperglycemic metabolism post-transplant has been described in recipients of a pancreatic transplant from an identical twin. These graft recipients however, did not obtain immunosuppressive drugs [16]. Usually high immunosuppression is supposed to prevent recurrence of type 1 diabetes. But in some reports a recurrence of disease has been documented despite sufficient immunosuppression [15,17].

The aim of this study was to investigate the prevalence of insulin and islet cell autoantibodies in a large series of pancreas graft recipients and to correlate antibodies with the endocrine function of the graft. Finally the influence or at least the association of immunosuppression on antibody existence will be analyzed.

Patients and methods

Study population

Ninety-one type 1 diabetic patients (50 male, 41 female) after SPK were investigated in a cross-sectional manner. SPK was performed in the Munich transplantation center. Patients underwent SPK between 1989 and 2004. Between 2003 and 2004 all graft recipients were screened for autoantibodies. Mean age at transplantation was 38 ± 7 years, duration of diabetes was 26 ± 7 years. Graft recipients were examined 79 ± 53 months after transplantation. The patients gave their informed consent.

All patients received ATG, a calcineurin-inhibitor, an antimetabolite and glucocorticoids as induction immunotherapy. For chronic immunosuppression patients received a calcineurin-inhibitor (cyclosporine or tacrolimus) as 'first-line' immunosuppression. Additionally mycophenolate mofetil (MMF) or azathioprine (AZA) was given. Only a few patients ($n = 22$) received low doses of glucocorticoids (methylprednisolone 2–4 mg/day) at the time of investigation. Eight patients received a duct-occluded segmental pancreas graft. Eighty-three patients received a whole pancreas graft either bladder-drained ($n = 35$) or enteric-drained ($n = 48$). All patients had systemic venous insulin drainage. At the time of the investigation all patients were insulin-free.

Analytical tests

The prevalence of autoantibodies to glutamate decarboxylase (GAD) and tyrosine phosphatase (IA-2) were analysed with a radiobinding assay (CentAK® anti-GAD65; CentAK® anti-IA-2; Mediapan Diagnostica, Selchow, Germany). The threshold for positivity was >0.9 U/ml for GAD autoantibodies and >0.75 U/ml for IA-2 autoantibodies. IAA were analyzed with a radiobinding assay as described by Ziegler *et al.* [18]. Screening for autoantibodies was performed after SPK, assessment of autoantibodies before transplantation was not available.

Graft recipients were grouped according to immunoreactivity. Group 1: no immunoreactivity; group 2: immunoreactivity to one antigen; group 3: immunoreactivity to two or three antigens.

Additionally parameters of graft function (fasting blood glucose, $HbA1_c$, oral glucose tolerance and serum creatinine) were measured. An automated glucose analyzer determined whole blood glucose levels; $HbA1_c$ levels were determined by high-performance liquid chromatography. An oral glucose tolerance test (OGTT) was performed after an overnight fast and without taking the immunosuppressants or other drugs at the time of testing. glucose of 100 g (i.e. 300 ml Dextro®-OGT; Roche Diagnostics, Mannheim, Germany) was ingested within 5 min. For blood sampling of glucose and insulin an intravenous line was placed, and blood samples were taken before (0 min) as well as 30 min, 60 min and 120 min after glucose load. According to the WHO criteria [19], the glucose tolerance test was defined as normal, if 2 h venous *whole* blood glucose was <120 mg/dl (6.7 mmol/l), as impaired between 120 and 180 mg/dl (6.7–11.1 mmol/l) and diabetic with 2 h blood values higher 180 mg/dl (11.1 mmol/l).

Calculations and statistical analysis

Data are expressed as mean ± SEM. Groups were compared by Kruskal–Wallis test followed by the Mann–Whitney *U*-test if appropriate. For categorical variables Fisher's exact test was used. $P < 0.05$ was considered to be statistically significant.

Results

Immunoreactivity

Antibodies to insulin were found in 44%, antibodies to GAD in 45% and antibodies against IA-2 were detected in 22% (Table 1). Twenty-five percent of the graft recipients did not show any immunoreactivity (group 1), 39% of patients displayed positivity for one antibody (group 2), whereas 36% were positive for two or three autoanti-

Table 1. Frequency of immunoreactivity to insulin, GAD and IA-2 in pancreas graft recipients.

Insulin antibodies (IAA)	51 (56)
Glutamate decarboxylase (GAD)	41 (45)
Tyrosine phosphatase (IA-2)	20 (22)
Group 1 (no immunoreactivity)	23 (25)
Group 2 (immunoreactivity to antigen)	35 (39)
Group 3 (immunoreactivity to two or three antigens)	33 (36)

Values are given as *n* (%).

Table 2. Clinical characteristic and laboratory parameters of graft recipients grouped to immunoreactivity.

	Group 1	Group 2	Group 3	*P*-value
Patients (*n*)	23	35	33	
Age at SPK (years)	39 ± 2	38 ± 1	39 ± 2	NS
Diabetes duration (years)	28 ± 2	26 ± 1	25 ± 2	NS
Time post-transplant (months)	75 ± 10	90 ± 10	70 ± 8	NS
S-creatinine (mg/dl)	1.8 ± 0.1	1.4 ± 0.1	1.8 ± 0.2	NS
Fasting blood glucose (mg/dl)	86 ± 11	90 ± 11	87 ± 13	NS
$HbA1_c$ (%)	5.6 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	NS
Normal glucose tolerance (%)	60	67	60	NS

Values are given as mean ± SEM.
Group 1, no immunoreactivity; group 2, immunoreactivity to one antigen; group 3, immunoreactivity to two or three antigens.

bodies (group 3). There was no relation of immunoreactivity and time after transplantation.

Correlation to graft function

Immunoreactivity was not associated with any aspect of pancreas graft function. There were no significant differences between the three groups of immunoreactivity regarding blood glucose or $HbA1_c$ values. Graft recipients, who were positive for one or more diabetes-specific antibodies, did not display more often an impaired or diabetic glucose tolerance (Table 2).

Immunosuppression

Glutamate decarboxylase
Concerning antibodies to GAD there were no differences between graft recipients treated with tacrolimus compared with recipients who received cyclosporine. Neither the type of antimetabolic immunosuppressant (MMF, AZA) nor the use of methylprednisolone had significant influence on the immunoreactivity for GAD (Table 3).

Insulin autoantibodies
Compared with patients treated with tacrolimus, graft recipients who received cyclosporine were significantly more often immunoreactive for insulin antibodies

Table 3. Immunoreactivity for autoantibodies (IAA, IA-2, GAD) in graft recipients grouped to immunosuppressive treatment.

	n	IAA+	GAD+	IA-2+
	91	51	41	20
CICL+	42	28 (67)*	19 (45)	13 (31)*
TAC+	49	23 (47)*	22 (44)	7 (14)
MMF+	69	35 (51)	30 (43)	15 (22)
AZA+	22	16 (72)	11 (50)	5 (23)
CICL+ MMF+	21	13 (62)	9 (43)	8 (38)
CICL+ AZA+	21	15 (71)	10 (48)	5 (24)
TAC+ MMF+	48	22 (46)	21 (44)	7 (15)
TAC+ AZA+	1	1	1	0
MPDN+	22	10 (45)	11 (50)	1 (5)***
MPDN–	69	41 (59)	30 (44)	19 (27)***
MPDN+ CICL+	11	6 (55)	5 (45)	1 (9)
MPDN+ TAC+	11	4 (36)	6 (54)	0
MPDN– CICL+	31	22 (71)	14 (45)	12 (38)**
MPDN– TAC+	38	19 (50)	16 (42)	7 (18)**
MPDN+ MMF+	14	5 (36)	7 (50)	0
MPDN+ AZA+	8	5 (62.5)	4 (50)	1 (12.5)

CICL+, TAC+, MMF+, AZA+ and MPDN+, treatment with cyclosporine, tacrolimus, mycophenolate mofetil, azathioprine and methylprednisolone at the time of investigation; MPDN–, no treatment with methylprednisolone at time of investigation; IA+, positivity for insulin autoantibodies; GAD+, positivity for GAD autoantibodies; IA-2+, positivity for IA-2 autoantibodies.
Percentage values are given in parentheses.
*$P < 0.05$ for tacrolimus versus cyclosporine.
**$P < 0.05$ for tacrolimus versus cyclosporine in patients without methylprednisolone.
***$P < 0.01$ for graft recipients with versus graft recipients without methylprednisolone.

(Table 3). IAA were more often positive in patients who did not receive methylprednisolone or MMF, although this was not significant.

Tyrosine phosphatase
Patients who were treated with cyclosporine were significantly more often immunoreactive for IA-2 in comparison with patients on tacrolimus (Table 3). Patients who did not take methylprednisolone were significantly more often positive for IA-2 antibodies. There was no difference between AZA and MMF (Table 3).

Discussion

Simultaneous pancreas-kidney transplantation is the treatment of choice in uremic type 1 diabetic patients [10,11]. Even pancreas transplantation alone is nearly exclusively performed in patients with type 1 diabetes. Type 1 diabetes is an autoimmune-mediated disease. Therefore recurrence of disease is at least possible after transplantation. Autoimmune phenomena or a recurrence of type 1 diabetes have been described [15–17]. Immunosuppressive

protocols applied in pancreas graft recipients are supposed to suppress the recurrence of disease [16].

While in islet transplantation graft failure occurs more often in autoantibody positive patients [20], the consequences of autoantibodies after pancreas transplantation are not clear [13,14].

In our study immunologic markers for type 1 diabetes were detected in more than the half of pancreas graft recipients despite immunosuppression. This was a cross-sectional study and the antibody status pretransplant is unfortunately lacking. Therefore it is not possible to decide, whether antibodies persist or have reappeared after transplantation. Immunoreactivity was not associated with impaired graft function. Blood glucose and $HbA1_c$ values as well as glucose disappearance after an OGTT were independent of the antibody status. Insulin secretion was not impaired in patients with positive autoantibodies. Thus 'present' immunoreactivity does not indicate a significant loss of intact β-cells.

As autoimmune insulitis probably requires time to impair glucose metabolism significantly, prospective investigations would be helpful to determine the influence of autoantibodies to graft function and graft survival more exactly. In a recent study the presence of autoantibodies before transplantation was not associated with later graft outcome [13]. However, a significant increase of antibody levels during the post-transplant follow-up was a marker of subsequent loss of pancreatic graft function [13].

Interestingly immunoreactivity differences between the immunosuppressive protocols were found. This observation has not been described so far. Patients who received cyclosporine as first-line immunosuppressant displayed more often positive autoantibodies for IA-2 and IAA than patients treated with tacrolimus. In addition, compared with graft recipients who were still on glucocorticoids, patients without steroid medication displayed more often immunoreactivity for IA-2. However these findings should be interpreted with caution. As there are no data concerning immunoreactivity before transplantation, it is not possible to describe a direct relation between antibody status and immunosuppression. We also cannot exclude an uneven distribution of patients with and without immunoreactivity in the tacrolimus or in the cyclosporine group. To investigate the effect on immunoreactivity of different immunosuppressive protocols exactly, a multivariate analysis could be more appropriate. However, this procedure is not allowed for analysis of categorical parameters. Therefore a subgroup analysis of different immunosuppressive regimen was performed (Table 3). The different results between tacrolimus and cyclosporine could be the consequence of differences in antimetabolite and glucocorticoid use. Nearly all patients with tacrolimus received MMF and not AZA. In addition steroid therapy was somewhat more frequent in patients with cyclosporine. However, in subgroup analyses there was no difference between MMF and AZA in patients who received cyclosporine. Steroid use was associated with a lower rate of immunoreactivity. The differences between the calcineurin inhibitors are therefore not explainable by a different frequency of glucocorticoid use.

At present an approximate prediction for the risk to develop type 1 diabetes mellitus is possible [21]. However, a safe and effective preventive therapy is missing. Immunosuppressive therapy is able to influence the course of autoimmune diabetes. It has been shown in newly diagnosed subjects with type 1 diabetes, that cyclosporine was successful in delaying sometimes even halting β-cell destruction [22,23]. It is speculative whether immunosuppression was responsible for negative antibody formation in our graft recipients without immunoreactivity.

Even if this was a cross-sectional study, the observation of uneven immunoreactivity in different immunosuppressive protocols is interesting and worthwhile for further investigations. Tacrolimus and cyclosporine are assumed to be diabetogenic and mainly responsible for post-transplant diabetes mellitus (PTDM), a frequent complication after transplantation. Nearly all studies have shown a higher incidence of PTDM for tacrolimus [24–26]. However, recently a risk factor analysis did not reveal a significant influence of immunosuppression [27]. After pancreas/kidney transplantation, parameters of glucose metabolism are not worse in patients receiving tacrolimus [28]. Pancreas graft survival is even better in patients treated with tacrolimus when compared with cyclosporine [29] and rejection episodes of pancreas grafts are reduced in patients who received tacrolimus [30]. From this data it is not possible to conclude a beneficial suppression of autoimmune recurrence by tacrolimus as explanation for better outcomes after pancreas transplantation. However further prospective investigations could solve this question.

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