Rapid Publications

Progressive Islet Graft Failure Occurs Significantly Earlier in Autoantibody-Positive Than in Autoantibody-Negative IDDM Recipients of Intrahepatic Islet Allografts

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Alloimmunity has been uncovered to be a cause of graft loss representing a major barrier for clinical islet transplantation, and several studies are designed to evaluate new strategies for immunosuppression to prevent alloimmunity. In contrast, the significance for autoimmune destruction of transplanted β-cells has remained somewhat controversial. Recently, two case reports based on histological findings have suggested recurrent autoimmune insulitis despite immunosuppressive therapy both in clinical pancreas and in islet transplantation. In the present study, in 23 islet-grafted patients with IDDM receiving standard immunosuppressive therapy, we demonstrate that progressive impairment of islet graft function occurs significantly earlier in those individuals positive for autoantibodies as a typical stigma of diabetes-associated autoimmunity that is well established in the prediabetic periods of IDDM. Intraportal infusion of allogeneic islets was performed in 23 C-peptide–negative IDDM patients, according to the clinical transplantation categories defined as islet after kidney (IAK) or simultaneous islet and kidney (SIK). Complete islet graft failure was defined as the 1st day of permanent C-peptide negativity in the serum (<0.2 ng/ml) and C-peptide negativity in the urine (<2 μg/dl). The median observation period following islet transplantation was 12 months (range 1–50) with a cumulative follow-up of 336 months. Islet cell antibodies (ICAs) and GAD65 antibodies were monitored before and regularly after islet transplantation. Kaplan-Meier survival analysis and log-rank statistics revealed a significant ($P < 0.05$) difference in cumulative islet graft survival depending on the presence of islet cell and/or GAD65 antibodies. These results strongly suggest that recurrent autoimmunity directed to transplanted β-cells contributes to islet graft failure despite sustained immunosuppression. For successful clinical islet transplantation in the future, new immunosuppressive therapies are needed to prevent both alloimmunity and autoimmunity. *Diabetes* 46:1907–1910, 1997

Recently, it has been hypothesized that alloimmunity plus autoimmunity represent principle barriers to successful islet transplantation (1). Two case reports have suggested recurrent autoimmune insulitis in both pancreatic and islet allografts. Tyden et al. (2) found selective β-cell destruction in two patients who had rejected their pancreatic grafts despite immunosuppression, with one of them generating islet cell antibodies (ICAs) and GAD65 antibodies after the transplantation. In accordance, Stegall et al. (3) also demonstrated selective β-cell destruction with lymphocytic infiltration in immunosuppressed islet-transplanted individuals, introducing the forearm subfascial site that gives the opportunity for repeated biopsies.

These case reports, based on histological findings, provide clear evidence of recurrent autoimmune insulitis despite sustained immunosuppression, and the results are consistent with our own observations of persistence and occurrence of autoantibodies in immunosuppressed IDDM patients who underwent islet transplantation (4). Autoantibodies such as ICAs or GAD65 antibodies are a hallmark of autoimmune diabetes and early markers for prediction in the prediabetic period (5). In clinical islet transplantation, multiple biopsies are not a suitable tool for morphological monitoring of intrahepatic islet grafts. We and others have shown that islet cell antibodies, and especially GAD65 antibodies, can persist or occur in pretransplant negative individuals despite sustained immunosuppression (4). Thus autoantibodies are obvious candidates for linking possible recurrent autoimmunity with islet graft function. The aim of the present study was to investigate the islet graft function in relation to the autoantibody status in 23 islet-transplanted patients with IDDM over a cumulative observation period of more than 28 patient-years post–islet transplantation.

RESEARCH DESIGN AND METHODS

Subjects. Participants in this study were patients with IDDM taking part in the Giessen islet transplantation project. All of the protocols were approved by the Justus-Liebig University ethics committee, and all subjects gave written informed consent before participation. A total of 23 patients were included from two recip-

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IAK, islet after kidney; ICA, islet cell antibody; ITX, islet transplantation; JDF, Juvenile Diabetes Foundation; SIK, simultaneous islet and kidney.

ient categories: islet after kidney transplantation (IAK, n = 12) and simultaneous islet and kidney transplantation (SIK, n = 11). The median age at the time of islet transplantation was 38 years (range 29–58) and the median duration of diabetes before islet transplantation was 24 years (range 12–38). A total of 12 males and 11 females were transplanted, and the median follow-up period posttransplantation was 12 months (range 1–50) with a cumulative follow-up of 336 months.

Islet transplantation. Islet allografts were transplanted intraportally according to the Giessen protocol as described elsewhere (6). All patients were pretransplant C-peptide negative. In the group of patients receiving combined kidney and islet transplants, immunosuppression was maintained by triple therapy (cyclosporine-A, steroids, azathioprine) in combination with an induction immunosuppression using antilymphocyte serum. The same regimen was followed in islet-transplanted patients with a previously established kidney graft, but in some cases, without administering azathioprine. Complete islet graft failure was defined as the 1st day of permanent C-peptide negativity in the serum (<0.2 ng/ml) and C-peptide negativity in a 24-h urine collection (<2 μg/dl). The median follow-up period posttransplantation was 12 months (range 1–50), with a cumulative follow-up of 336 months, giving an observation period post–islet transplantation of 28 patient-years.

Serum and autoantibodies. In all cases, at least one pretransplant serum sample was available for assessment of ICAs and GAD65 antibodies before islet transplantation. All patients regularly attended the follow-up visits after the islet transplantation for further monitoring of autoantibodies and evaluation of islet graft function. GAD65 antibodies were detected in a radioligand GAD65 Ab assay, using recombinant, in vitro translated, human islet [^{35}S]methionin-labeled GAD65 as tracer according to the protocol developed by Peterson et al. (7). Antibody levels were expressed as index values: GAD65 antibody index = (cpm unknown sample – cpm negative standard serum)/(cpm positive standard serum – cpm negative standard serum). The cDNA encoding for human GAD65 was generously donated by Novo Nordisk A/S, Denmark. The cutoff index level for GAD65 antibody positivity was determined from 100 healthy control subjects. Sera with GAD65 antibody index values above the mean index plus two times SD were regarded as positive. This assay has been evaluated in the International Diabetes Workshop (IDW) proficiency workshop series on standardization of GADA, performing with 91% specificity and 86% sensitivity. ICAs were determined by indirect immunofluorescence technique using cryostat sections of human pancreas as substrate. This assay is regularly tested in the ENDIT quality control workshops and in the IDW proficiency workshop series on standardization of the islet cell anitbody assay, performing with 100% sensitivity and 100% specificity. Titers have been converted to Juvenile Diabetes Foundation (JDF) units using a JDF standard reference serum. The detection limit of the assay is 5 JDF units.

Statistical analysis. The islet graft survival was analyzed by Kaplan-Meier survival analysis for data containing censored observations. Because of the high percentage of censored observations, we used log-rank statistics to determine statistically significant differences of islet graft survival relative to the autoantibody status, and a P value of <0.05 was considered significant. The calculations were carried out using SPSS 6.1.3 software.

RESULTS

Autoantibodies. Focusing on GAD65 antibodies, we observed positivity in 11 out of 23 (48%) individuals. Eight patients were positive for GAD65 antibodies before islet transplantation, and three subjects seroconverted subsequently to transplantation from GAD65 antibody negativity to positivity. For islet cell antibodies, the prevalence was lower with 5 out of 23 (21%) patients generating ICAs. Three subjects were pretransplant positive for ICAs, and only in two cases did we observe seroconversion afterward.

Looking at both autoantibodies together in our study population, two individuals were double-positive before islet transplantation, and one patient showed seroconversion for both antibody specificities after islet transplantation. In two other cases, we observed pretransplant positivity for one type of autoantibody with seroconversion of the other specificity following the islet transplantation. Thus, each of the five ICA positive individuals was additionally double-positive also for GAD65 antibodies either before or subsequent to islet transplantation, with none of the patients being exclusively positive for ICAs without GAD65 antibodies.

Taken together, we observed generations of autoantibodies either pretransplantation and/or posttransplantation in 11 out of 23 (48%) IDDM recipients of intrahepatic islet allografts. The clinical characteristics of the autoantibody positive versus autoantibody negative individuals did not differ regarding age at the time of islet transplantation (ITX), diabetes duration before ITX, or the sex distribution (Table 1).

Islet graft survival. According to our definition of complete islet graft failure, we observed ongoing graft function in 17 out of 23 (74%) islet-transplanted individuals during the observation period. Complete islet graft failure was found in 6 out of 23 (26%) patients after 1, 2, 3, 4, 7 and 10 months, respectively. Interestingly, 5 out of 6 (83%) patients with complete graft failure belonged to the group of autoantibody-positive individuals (Table 1).

Kaplan-Meier estimate. Figure 1 shows the cumulative islet graft survival of the autoantibody positive patients (n = 11) who developed either GAD65 antibodies or both antibodies (GAD65 and ICA) during the observation period, compared with those individuals (n = 12) who did not generate GAD65 antibodies or ICAs at any time pre– and/or post–islet transplantation. Log-rank statistics revealed a significant (P < 0.05) difference of islet graft survival in regard to the autoantibody status, demonstrating a significantly lower graft survival in the group of autoantibody-positive IDDM recipients of intrahepatic islet allografts.

DISCUSSION

Since the landmark paper of Sibley et al. (8) from the Sutherland group in 1985, it has been accepted that in the case of identical twin pancreatic transplants, recurrent autoimmune β-cell destruction may occur in patients with IDDM, unless cyclosporine and azathioprine immunosuppression are used. Recently, the dogma that immunosuppressive therapy required to prevent alloimmunity is sufficient for the prevention of autoimmunity has been disputed for both pancreatic and islet transplantation. Two case reports based on histological analysis provided evidence of recurrent autoimmunity in human allogeneic islet transplantation (3), and a few months later another report suggested recurrence of autoimmune diabetes in recipients of cadaveric pancreatic grafts (2). In both studies, typical histological signs of autoimmune insulitis with selective destruction of β-cells and a relative sparing of α- and δ-cells had occurred despite sustained immunosuppression.

TABLE 1
Clinical characteristics of 23 IDDM recipients of intrahepatic islet allografts grouped by islet cell and/or GA65 antibody positivity versus autoantibody negativity

	Autoantibody-positive patients	Autoantibody-negative patients
n	11	12
Age at the time of ITX		
Median (years)	39	36
Range	29–58	32–54
Sex (M/F)	7/4	5/7
DD before ITX	24 (15–38)	23 (12–38)
Cases of complete islet graft failure	5	1

Data are median years (range) or n. DD, diabetes duration; ITX, islet transplantation.

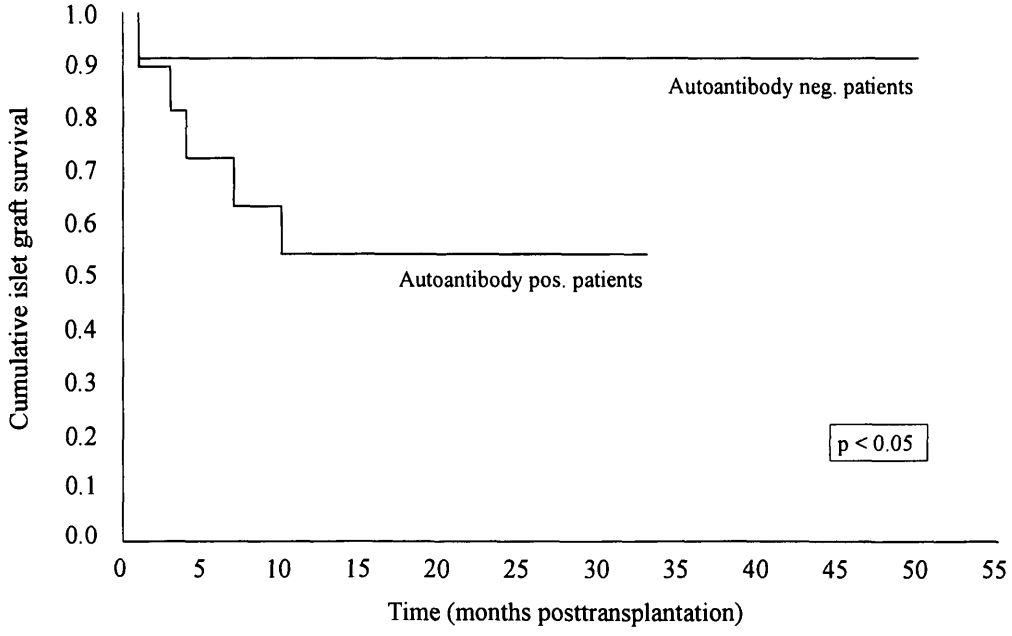

FIG. 1. Kaplan-Meier estimate of the cumulative islet graft survival depending on the presence of GAD65 and/or islet cell antibodies. $P < 0.05$ for the group of autoantibody positive ($n = 11$) versus autoantibody negative ($n = 12$) patients with IDDM receiving an intrahepatic islet allograft.

The histological approach provides strong evidence for the underlying pathophysiological process but is not widely applicable in the clinical situation, at least for intrahepatic islet allografts. Therefore, we aimed for alternative tools to identify recipients with IDDM at risk for recurrent autoimmunity. Keeping in mind what has been learned from prediabetic periods of IDDM, the presence of humoral autoimmunity with generation of different autoantibodies such as ICAs, GAD65 antibodies, or IA-2 antibodies offers the opportunity to identify individuals with a high risk for developing IDDM at a very early stage several years before clinically overt disease (5). Interestingly, it has been shown in clinical trials of recent onset of IDDM that GAD65 antibodies are not affected by immunosuppressive therapy with cyclosporine-A, in contrast to significantly reduced ICAs and insulin autoantibodies (9,10). Recently, we transferred these observations to the situation of clinical islet transplantation, demonstrating that GAD65 antibodies and, to a lesser degree, ICAs can persist despite sustained immunosuppression or occur in pretransplant autoantibody-negative islet-transplanted IDDM patients. In addition, we performed a pilot study on seven IAK-transplanted IDDM patients suggesting that the presence of GAD65 antibodies may adversely affect islet graft function (4).

The worldwide experience of sustained functional survival of islet allografts is based on a relatively small, albeit an increasing, number of cases. The present study included 23 IDDM recipients of intrahepatic islet allografts with a cumulative observation period of 336 months posttransplantation, thus substantially expanding our previous findings. We have now been able to confirm our previous observations of persistence and occurrence of autoantibodies despite immunosuppression, obtained from seven islet transplanted individuals (4) in the larger cohort of 23 IDDM recipients of intrahepatic islet allografts. Furthermore, the present study indicates that detection of autoantibodies might become a useful tool to identify individuals in whom progressive islet graft failure occurs significantly earlier, compared with autoantibody-negative subjects. In this study five out of six patients who had suffered complete islet graft failure generated autoantibodies either before or after islet transplantation. The individual transplant survival times of our recipient cohort to date by means of lifetable procedure and log-rank statistics demonstrate for the first time a statistically significant difference ($P < 0.05$) in islet allograft survival depending on the presence of autoimmune phenomena, such as GAD65 antibodies and ICAs.

We conclude that the work of Tyden et al. (2) and Stegall et al. (3), together with the results of our present study, has created several lines of evidence for recurrent autoimmunity directed to transplanted β-cells despite standard immunosuppressive therapy. Detection of autoantibodies may help to identify the individuals at high risk for recurrent autoimmunity. Finally, new immunosuppressive strategies that prevent both alloimmunity and autoimmunity are needed to ensure successful clinical islet transplantation in the future.

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