METABOLIC AND IMMUNOLOGICAL FEATURES OF THE FAILING ISLET TRANSPLANTED PATIENT

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ABSTRACT

Objective: This retrospective study was designed to identify metabolic and immune predictors of early islet allograft failure.

Research Design and Methods: We measured several metabolic and immunological markers at the time of pre-transplant and several time points post-transplantation in 17 patients with long-term functioning graft (Long fx) and 20 patients with short-term functioning graft (Short fx).

Results: The Short fx group, but not the Long fx one, showed higher insulin resistance, altered pro-insulin processing, lower sIL2-r (marker of T cell activation), and higher sFasL (marker of apoptosis) during the entire follow-up particularly at time of failure.

Conclusions: Patients who experienced an early failure of islet allograft showed specific metabolic and immunological signs long before islet failure.
Despite recent progress in islet transplantation, the rate of islet failure is still high, and insulin independence in all transplanted patients has not been achieved. While it is clear that the partial secretory ability conferred by islet transplantation helps halt the progression of diabetic complications, little is known regarding the mechanisms of islet graft failure.

We studied metabolic and immune peripheral markers in patients, whose transplanted islets failed at early time points, aiming to detect a panel of markers that could aid early diagnosis of islet dysfunction.

**RESEARCH DESIGN AND METHODS**

Patients were retrospectively split into 17 with long-term functioning graft (Long fx) (C-peptide >1.0 ng/ml for more than 12 months) and 20 patients with short-term functioning graft (Short fx) (C-peptide levels <1 ng/ml within 12 months of islet transplantation). Please see the online appendix (available at http://care.diabetesjournals.org) for a full description of the Methods.

Blood samples were collected from these patients on a monthly basis after islet transplantation. We measured metabolic and immune peripheral markers obtained at baseline (before islet transplantation); from a second time point at the peak of transplanted islet function (defined by the highest C-peptide level) (Short fx=4±1 vs. Long fx=11±2 months), and at the time of failure in the Short fx group (9±1 months) or at the latest time point available in the Long fx group (24±4 months).

**RESULTS**

The two groups of patients appeared similar regarding baseline characteristics (Table 1, Online appendix).

1. Metabolic processes
   1a. Islet function

The Long fx group showed higher C-peptide levels (P<0.01 at both time points), lower exogenous insulin requirement (P=0.04 and P=0.01 at the second and third time points, respectively) and lower glycated hemoglobin compared to the Short fx group (Figure 1A and 1B and data not shown).

1b. Islet mass and insulin secretory reserve

The number of islets was similar in the two groups (Equivalent number: Short fx=542,776±63,606 vs. Long fx=585,600±59,481, ns; Table 1, Online Appendix), excluding a potential bias due to a different number of transplanted islets. Furthermore, a L-arginine test performed one month after islet transplantation, showed a similar insulin secretory reserve in the two groups (area under the curve of insulin release: Short fx=2674±516 vs. Long fx=2772±338, ns).

1c. Insulin processing and islet overworking

A higher proinsulin/C-peptide ratio was evident in the Short fx group, particularly at the third sampling (failure vs. long term function) (Short fx=32.4±11.9 vs. Long fx=7.0±1.6, P=0.04), (Figures 1C-1D). These data suggest a disproportion between the amount of proinsulin produced and the amount processed. Activin-A, which is considered a good marker of α-cell activity, being co-secreted with glucagons, was slightly higher in the Short fx group than in the Long fx group (ns, data not shown). Amylin levels, as a marker of fibrillogenesis, did not differ between the 2 groups (data not shown).

1d. Insulin resistance (HOMA-IR)

HOMA-IR (Homeostasis model assessment of insulin resistance) has been previously validated in kidney-transplanted patients to be one of the most reliable methods to assess insulin resistance. HOMA-IR scores were similar in the two groups at the time of islet transplantation, indicating the groups' homogeneity. HOMA-IR was higher in the Short fx group than in the Long fx group at
the peak of islet function (7.5±2.3 vs. 3.5±0.5, respectively, P=0.02), (Figures 1E-1F), indicating that insulin resistance is already present in the Short fx group long before the failure of islets.

2. Immune processes and apoptotic processes

2a. Alloimmune response
During follow-up, soluble IL-2r (sIL2r) was higher in the Long fx group, particularly at the third time point (Long fx=4901±1033 vs. Short fx=2754±490 pg/ml, P=0.04), (Figures 1G-1H). sIL-2r is a marker of T cell activation, released peripherally during any immunological process, but it has a role in modulating CD4⁺CD25⁺ (Tregs) cells’ function. The persistence of high sIL2r levels suggests a higher percentage of CD4⁺CD25⁺ cells with regulatory ability releasing the soluble form of the receptor that they expressed (CD25 is IL2r). High sIL2r levels could also be a sign of some activation/tolerogenic processes.

2b. Autoimmune response
High levels of autoantibodies are associated with increased failure of the islet graft. A rise in IA-2As (autoantibodies to protein tyrosine phosphatase isoforms IA-2) was observed in the Short fx group (Short fx: 1st time point=1.07±0.62 vs. 2nd time point=15.45±13.94 AU, p<0.05; Long fx: 1st time point=15.45±13.94 AU, p<0.05; Long fx: 2nd time point=2.52±1.21 vs. 2nd time point=2.37±1.30 AU, ns) but not in the Long fx group (data not shown). GADAs (antibodies to glutamic acid decarboxylase) also increased in the Short fx group, although without reaching statistical significance.
It is not known whether members of the Long fx group are simply less prone to the recurrence of autoimmunity or are more immunosuppressed, but it is generally accepted that the immunosuppressive regimens (including cyclosporine) currently used in islet transplantation are not specific for autoimmunity.

3. Apoptotic processes
3a. sFasL release: None of the patients showed detectable levels of peripheral Annexin V (data not shown), but sFasL (a marker of apoptotic process) was higher in the Short fx group at the earlier time point, when islet function was peaking. An increase in sFasL levels compared to baseline was particularly evident in the Short fx group (from 0.570±0.422 at baseline to 1.862±1.639 at islet function peak, p<0.05). This was not seen in the Long fx group, where sFasL was more stable (from 0.356±0.136 at baseline to 0.706±0.424 at islet function peak). This increase was maintained even at the 3rd time point of follow-up (Short fx group=0.701±0.452 vs. Long fx group=0.387±0.161). There were no differences in sFas levels between the two groups (data not shown).

CONCLUSIONS
In our study, increased insulin resistance and altered insulin processing are evident before the failure of the graft. Metabolic and immunological markers could help in identifying patients at high risk for early graft failure, indicating that immunological phenomena also predict the failure of the islets.

Metabolic and immunological markers could help in identifying patients at high risk for early graft failure. Our data can help to define early markers that could be used as routine tests to identify or predict islets rejection.
REFERENCES

FIGURE LEGEND

Figure 1. Peripheral markers of islet function. Peripheral markers of islet function were evaluated in the *Short fx* group (n=20 patients) and in the Long fx group (n=17 patients). Fasting C-peptide assessment in the 2 groups revealed that at the peak of function the *Short fx* group already had reduced C-peptide levels (*P<0.01 at second and third time point compared to Long fx group), Panel A). The Long fx group showed stable β-cell endocrine function, with an improvement in glucose control, as shown by the reduced EIR (#P=0.04 at second and **P=0.01 at third time point compared to Long fx group), Panel B). A higher proinsulin/C-peptide ratio was evident in the Short fx group particularly at the time of failure (Panels C and D, p=0.04). HOMA-IR (Homeostasis model assessment of insulin resistance) (Panels E and F), was higher in the Short fx group, even at the peak of function (p=0.02 compared with the time of failure). The Long fx group showed a persistent increase in soluble IL2 receptor (sIL2r) with a peak at the third time point (Panels G and H), (p=0.04).
FIGURE 1

A

B

C

D

E

F

G

H

FIGURE 1