

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. **Error! Reference source not found.** While it is clear that the partial secretory ability conferred by islet transplantation helps halt the progression of diabetic complications. **Error! Reference source not found.**, little is known regarding the mechanisms of islet graft failure. **Error! Reference source not found.**

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. **Error! Reference source not found.**, 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. **Error! Reference source not found.** 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 **Error! Reference source not found.** 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= 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 **Error! Reference source not found.**

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 **Error! Reference source not found.** The early failing group showed high levels of sFasL, low levels of sIL2r, and a rise in autoantibody titres **Error! Reference source not found.**, 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 **Error! Reference source not found.** Our data can help to define early markers that could be used as routine tests to identify or predict islets rejection.

REFERENCES

1. Ricordi C. Islet transplantation: a brave new world. *Diabetes*. 2003;52:1595-1603.
2. Ryan EA, Lakey JR, Rajotte RV, et al. Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. *Diabetes*. 2001;50:710-719.
3. Ryan EA, Paty BW, Senior PA, et al. Five-year follow-up after clinical islet transplantation. *Diabetes*. 2005;54:2060-2069.
4. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med*. 2000;343:230-238.
5. Shapiro AM, Nanji SA, Lakey JR. Clinical islet transplant: current and future directions towards tolerance. *Immunol Rev*. 2003;196:219-236.
6. Hering BJ, Kandaswamy R, Ansite JD, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *Jama*. 2005;293:830-835.
7. Fiorina P, Folli F, Bertuzzi F, et al. Long-term beneficial effect of islet transplantation on diabetic macro-/microangiopathy in type 1 diabetic kidney-transplanted patients. *Diabetes Care*. 2003;26:1129-1136.
8. Fiorina P, Folli F, Maffi P, et al. Islet transplantation improves vascular diabetic complications in patients with diabetes who underwent kidney transplantation: a comparison between kidney-pancreas and kidney-alone transplantation. *Transplantation*. 2003;75:1296-1301.
9. Fiorina P, Folli F, Zerbini G, et al. Islet transplantation is associated with improvement of renal function among uremic patients with type I diabetes mellitus and kidney transplants. *J Am Soc Nephrol*. 2003;14:2150-2158.
10. Fiorina P, Gremizzi C, Maffi P, et al. Islet transplantation is associated with an improvement of cardiovascular function in type 1 diabetic kidney transplant patients. *Diabetes Care*. 2005;28:1358-1365.
11. Davalli AM, Ogawa Y, Scaglia L, et al. Function, mass, and replication of porcine and rat islets transplanted into diabetic nude mice. *Diabetes*. 1995;44:104-111.
12. Braghi S, Bonifacio E, Secchi A, Di Carlo V, Pozza G, Bosi E. Modulation of humoral islet autoimmunity by pancreas allotransplantation influences allograft outcome in patients with type 1 diabetes. *Diabetes*. 2000;49:218-224.
13. Ricordi C, Strom TB. Clinical islet transplantation: advances and immunological challenges. *Nat Rev Immunol*. 2004;4:259-268.
14. Shapiro AM, Ricordi C. Unraveling the secrets of single donor success in islet transplantation. *Am J Transplant*. 2004;4:295-298.
15. Kenyon NS, Chatzipetrou M, Masetti M, et al. Long-term survival and function of intrahepatic islet allografts in rhesus monkeys treated with humanized anti-CD154. *Proc Natl Acad Sci U S A*. 1999;96:8132-8137.
16. Yasuda H, Inoue K, Shibata H, et al. Existence of activin-A in A- and D-cells of rat pancreatic islet. *Endocrinology*. 1993;133:624-630.
17. Ogawa K, Abe K, Kurosawa N, et al. Expression of alpha, beta A and beta B subunits of inhibin or activin and follistatin in rat pancreatic islets. *FEBS Lett*. 1993;319:217-220.
18. Perseghin G, Caumo A, Sereni LP, Battezzati A, Luzi L. Fasting blood sample-based assessment of insulin sensitivity in kidney-pancreas-transplanted patients. *Diabetes Care*. 2002;25:2207-2211.
19. Martin S, Pawlowski B, Greulich B, Ziegler AG, Mandrup-Poulsen T, Mahon J. Natural course of remission in IDDM during 1st yr after diagnosis. *Diabetes Care*. 1992;15:66-74.

20. Finzi G, Davalli A, Placidi C, et al. Morphological and ultrastructural features of human islet grafts performed in diabetic nude mice. *Ultrastruct Pathol.* 2005;29:525-533.
21. Ryan EA, Lakey JR, Paty BW, et al. Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes.* 2002;51:2148-2157.
22. Jaeger C, Brendel MD, Hering BJ, Eckhard M, Bretzel RG. Progressive islet graft failure occurs significantly earlier in autoantibody-positive than in autoantibody-negative IDDM recipients of intrahepatic islet allografts. *Diabetes.* 1997;46:1907-1910.
23. Han D, Xu X, Baidal D, et al. Assessment of cytotoxic lymphocyte gene expression in the peripheral blood of human islet allograft recipients: elevation precedes clinical evidence of rejection. *Diabetes.* 2004;53:2281-2290.
24. Han D, Xu X, Pastori RL, Ricordi C, Kenyon NS. Elevation of cytotoxic lymphocyte gene expression is predictive of islet allograft rejection in nonhuman primates. *Diabetes.* 2002;51:562-566.

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

