Metabolic and Immunological Features of the Failing Islet-Transplanted Patient

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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 pretransplant and several time points posttransplantation 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 showed higher insulin resistance, altered proinsulin processing, lower soluble interleukin-2 receptor (sIL-2r) (marker of T-cell activation), and higher soluble FasL (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.

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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 (1–6). While it is clear that the partial secretory ability conferred by islet transplantation helps halt the progression of diabetes complications (7–10), little is known regarding the mechanisms of islet graft failure (11–15). 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 two groups: 17 patients 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 <1.0 ng/ml within 12 months of islet transplantation). Please see the online appendix (available at http://dx.doi.org/10.2337/dc07-1831) for a full description of 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: mean ± SE 4 ± 1 vs. 11 ± 2 months for patients with short fx and long fx, respectively), and at 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 (supplemental Table).

Metabolic processes

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 with that of the short fx group (Fig. 1*A* and *B* and data not shown).

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 [supplemental Table]), excluding a potential bias due to a different number of transplanted islets. Furthermore, an L-arginine test performed 1 month after islet transplantation showed a similar insulin secretory reserve in the two groups (area under the curve of insulin release 2,674 ± 516 vs. 2,772 ± 338 for short fx vs. long fx; NS).

Insulin processing and islet overworking. A higher proinsulin–to–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; $P = 0.04$), (Fig. 1*C* and *D*). These data suggest a disproportion between the amount of proinsulin produced and the amount processed. Levels of activin-A, which is considered a good marker of α-cell activity because it is cosecreted with glucagons (16,17), were slightly higher in the short fx group than in the long fx group (NS, data not shown). Amylin level as a marker of fibrillogenesis did not differ between the two groups (data not shown).

Insulin resistance. Homeostasis model assessment of insulin resistance (HOMA-IR) has been previously validated in kidney-transplanted patients as one of the most reliable methods to assess insulin resistance (18). HOMA-IR scores were similar in the two groups at the time of islet

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Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; long fx, long-term functioning graft; sFasL, soluble FasL; short fx, short-term functioning graft; sIL-2r, soluble interleukin-2 receptor.

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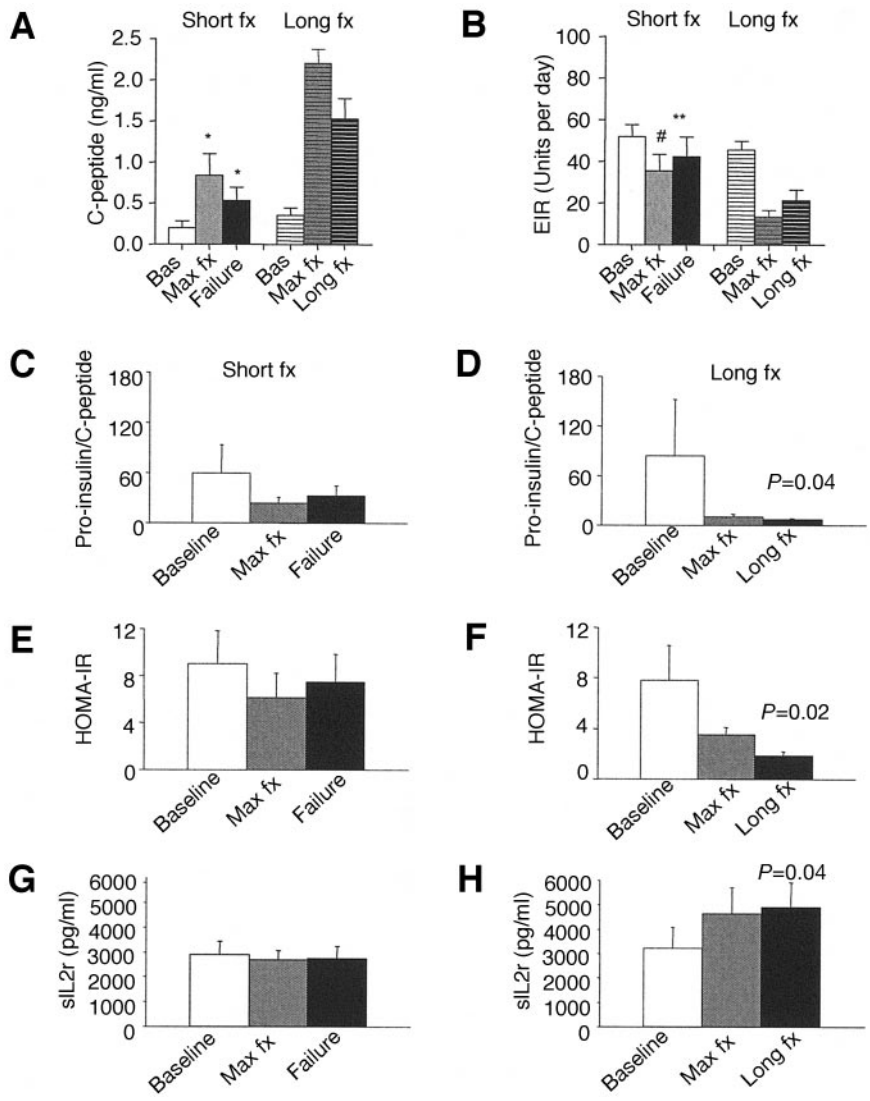

Figure 1—*Peripheral markers of islet function. Peripheral markers of islet function were evaluated in the short fx* (n = *20 patients*) *and long fx* (n = *17 patients*) *groups. Fasting C-peptide assessment in the two groups revealed that at the peak of function, the short fx group already had reduced C-peptide levels* (*P <0.01 *at second and third time point compared to long fx group*). A: *The long fx group showed stable β-cell endocrine function with an improvement in glucose control, as shown by the reduced exogenous insulin requirement* (#P = *0.04 at second and* **P = *0.01 at third time point compared with long fx group*). B: *A higher proinsulin–to–C-peptide ratio was evident in the short fx group, particularly at time of failure* (C *and* D *[*P = *0.04]*). E *and* F: *HOMA-IR was higher in the short fx group, even at the peak of function* (P = *0.02 compared with time of failure*). *The long fx group showed a persistent increase in sIL-2r with a peak at the third time point* (G *and* H, P = *0.04*).

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, $P = 0.02$) (Fig. 1*E* and *F*), indicating that insulin resistance is already present in the short fx group long before failure of islets.

Immune processes and apoptotic processes

Alloimmune response. During follow-up, sIL-2r was higher in the long fx group, particularly at the third time point (4,901 ± 1,033 vs. 2,754 ± 490 pg/ml for long fx and short fx, respectively; $P = 0.04$) (Fig. 1*G* and *H*). sIL-2r is a marker of T-cell activation, released peripherally during any immunological process, but it has a role in modulating $CD4^+CD25^+$ regulatory T-cells' function. The persistence of high sIL-2r levels suggests a higher percentage of $CD4^+CD25^+$ cells with regulatory ability releasing the soluble form of the receptor that they expressed (CD25 is an interleukin-2 receptor). High sIL-2r levels could also be a sign of some activation/tolerogenic processes.

Autoimmune response. High levels of autoantibodies are associated with increased failure of the islet graft (12). A rise in autoantibodies to protein tyrosine phosphatase isoforms IA-2 was observed in the short fx group (short fx first time point 1.07 ± 0.62 arbitrary units [AU] vs. second time point 15.45 ± 13.94 AU, $P < 0.05$; long fx first time point 2.52 ± 1.21 AU vs. second time point 2.37 ± 1.30 AU, NS) but not in the long fx group (data not shown). Antibodies to glutamic acid decarboxylase also increased in the short fx group, though 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 (19).

Apoptotic processes

Soluble FasL release. No patients showed detectable levels of peripheral annexin V (data not shown), but soluble FasL (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 was particularly evident in the short fx group (from 0.570 ± 0.422 ng/ml at baseline to 1.862 ± 1.639 ng/ml 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 ng/ml at baseline to 0.706 ± 0.424 ng/ml at islet function peak). This increase was maintained even at the third time point of follow-up (short fx group 0.701 ± 0.452 ng/ml vs. long fx group 0.387 ± 0.161 ng/ml). There were no differences in sFasL levels between the two groups (data not shown).

CONCLUSIONS

In our study, increased insulin resistance and altered insulin processing are evident before failure of the graft (2,3,11,20,21). The early failing group showed high levels of sFasL, low levels of sIL-2r, and a rise in autoantibody titers (12,22), indicating that immunological phenomena also predict failure of islets.

Metabolic and immunological markers could help in identifying patients at high risk for early graft failure (23,24). Our data can help to define early markers

that could be used as routine tests to identify or predict islet rejection.

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