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Studies of Circulating Microparticle Release in Peripheral Blood After Pancreatic Islet Transplantation

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ABSTRACT

The loss of graft function after intraportal islet transplantation is likely multifactorial involving allogeneic rejection, recurrent autoimmunity, graft exhaustion due to a marginally implanted islet mass, immunosuppressant toxicity, and impaired β-cell regeneration. Because early markers of the loss of β-cell mass or function are lacking, monitoring of islet function remains a challenging issue. We have reported herein monitoring of membrane procoagulant microparticles (MPs) as markers of cell stress in the plasma of three recipients with various clinical histories. Early kinetics of C-peptide and MPs followed identical patterns during the first weeks after transplantation; a major increase probably reflected processes related to cell infusion and islet engraftment. Importantly in the case of rejection, MPs and C-peptide showed opposite patterns. A fall in C-peptide was associated with enhanced insulin needs. Our results suggested that a peak in MP levels might indicate rejection with prognotic value. Treatment of the loss of islet function by a new islet infusion or steroid therapy returned MP and C-peptide levels to their baselines with concomitant restoration of islet function. In the patient with suspected acute cellular rejection, MPs also appeared to be sensors of immunosuppressive steroid therapy.

ALLOGENEIC ISLET TRANSPLANTATION can restore insulin secretion in patients suffering type 1 diabetes. However, the majority of recipients experience a gradual decline in graft function,[1] likely of multifactorial causes: allogenic rejection, recurrent autoimmunity, graft exhaustion due to a marginally implanted islet mass, immunosuppressant toxicity, and impaired β-cell regeneration. It is, however, often difficult to delineate a specific mechanism in each individual.

The development of de novo antibodies against human leukocyte antigen (HLA) class I and II molecules has been reported among subjects with failed islet transplantations[2]; graft loss can coincide with sensitization to donor HLA factors.[3] Recently, we reported recovery of islet graft function after treatment with an anti-CD20 monoclonal antibody and intravenous immunoglobulins.[4] Various markers and modalities have been assessed for early identification and treatment of harmful immunologic events: granzyme B, insulin mRNA, and (soluble CD30).[5–9] At the present time, none of them has gained wide clinical recognition and applicability. Indeed, the lack of circulating markers and the low islet sampling rate by needle biopsy after cellular rejection hamper detection of graft loss.[10]

Procoagulant microparticles (MPs) are submicron plasma membrane fragments shed from cells after stimulation or apoptosis.[11] Because the quantity of MPs is proportional to the degree of apoptosis or to the stress intensity, they are considered to be markers of cell death or stimulation.[12,13] MPs expose phosphatidylserine (PhtdSer), a procoagulant phospholipid translocated from the inner to the outer

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leaflet of the plasma membrane in response to stress. Tissue factor (TF) is another procoagulant moiety that circulates in its active form when exposed by MPs.[14] MPs also convey a broad spectrum of bioactive molecules believed to contribute to cell cross talk.[15,16] In clinical settings, MPs accessible in venous blood are measured at low levels in healthy individuals. Circulating MPs are indicators of the severity of illness and with prognosis value among patients at risk of thrombosis[17]; in type 1 diabetes, they circulate at high levels.[18]

The utility of circulating MPs as indicators of tissue apoptosis has been suggested in HIV patients and in models of atherothrombosis.[12,16] Rejection is another situation in which tissues are at risk of stress.[19] Recent data showing that circulating MPs of distinct cell origin are early independent makers of a first acute rejection episode in heart transplant patients suggest their possible use to monitor kidney recipients.[20,21] In islet transplantation, mechanisms of tissue remodeling and cell interactions during engraftment remain poorly understood. Early inflammatory, oxidative and apoptotic stresses have been described, and MPs bearing TF have been detected in situ during the instant blood-mediated immune reaction at 24 hours after islet infusion.[22]

MPs, as markers of cell death detected after rejection episodes, may conversely indicate restoration of graft function after immunosuppressive therapy. We monitored circulating MPs as markers of β-cell mass in three islet transplant patients with various clinical issues. Total plasma MPs were measured using a functional assay based on their PhtdSer content for correlation with metabolic markers of islet function.[23,24]

BLOOD SAMPLING AND MICROPARTICLE MEASUREMENT BY PROTHROMBINASE ASSAY

Peripheral blood collected in 0.129 mol/L sodium citrate before and after transplantation was processed as previously recommended.[23,24] Briefly, platelet-free plasma (PFP) containing MPs were obtained by two centrifugation steps (1500*g*/15 min and 14000*g*/2 min) was frozen (−80°C) until analysis. Functional assessment of total MPs in PFP was performed in duplicate using a solid-phase capture system based on the high affinity of unsolubilized annexin 5 for PhtdSer exposed by MPs combined with a prothrombinase assay.[23,25] The assay measured the PhtdSer content of MPs by measuring the promoted activation of prothrombin to thrombin in a calibrated purified system. Generated thrombin was assessed by changes in linear absorbance resulting from the cleavage of a chromogenic substrate (PNAPEP, CRYOPOPEP, France). Results are expressed as PhtdSer equivalents (PhtdSerEq) by reference to a standard curve constructed with liposomes of known PhtdSer concentrations.

CASE REPORTS (FIGURE 1)

All patients were enrolled in the GRAGIL (patients 1 and 2) or in the GRAGIL-TRIMECO trials (patient 3) undergoing islet transplantation by percutaneous intraportal infusion for metabolic instability. Informed consent was obtained; the investigation was approved by local Medical Ethics Committees (CPP, Grenoble, France).

Patients 1 and 2 were a 57-year-old man and a 41-year-old women with 43- and 36-year histories of type 1 diabetes, respectively. Immunosuppression was based on sirolimus (Rapamune, Wyeth; 0.1 mg/kg/d; target trough levels of 12–15 μg/L) and tacrolimus (Prograf, Fujisawa; 2 mg/d; target trough levels of 4–6 μg/L) with daclizumab induction (Zenapax, Roche; 1 mg/kg, five doses).

Patient 1 received 5420 islet equivalents (IEQ)/kg from a single donor. One week after transplantation, the C-peptide plasma level had increased to 3.48 ng/mL and returned to 1.67 ng/ml. Interestingly (Fig 1), MPs followed an identical pattern, increasing from 6.8 nmol/L to 14.2 nmol/L PhtdSerEq immediately after transplantation and returning to 7.5 nmol/L PhtdSerEq. Two months later, the islet graft was functional with baseline values C-peptide 1.21 ng/mL and MPs of 9.3 PhtdSerEq and a decrease in exogenous insulin requirement from 40 IU/d to 6 IU/d.

Patient 2 received three successive islet infusions of 4070 IEQ/kg, 6073 IEQ/kg, and 7076 IEQ/kg. After the first two infusions, C-peptide plasma levels increased to 1.18 ng/mL with MP level at 8.4 nmol/L PhtdSerEq remaining stable over 10 months. The peak MPs reached 18.6 nmol/L PhtdSerEq at 2 months before the loss of islet function as reflected by an abrupt drop in C-peptide and increase in insulin needs with hypoglycemic events. After the third islet injection, C-peptide as well as MPs returned to their baseline values (1.8 ng/mL); exogenous insulin needs decreased.

Patient 3 was a 43–year-old woman with a 37-year history of diabetes, coronary disease, and stent placement. A search for HLA antibodies by Luminex was consistently negative for HLA classes I and II. The patient received 7400 IEQ/kg from a single donor. Pretransplant complement-dependent crossmatches using donor T and B lymphocytes and microlymphocytotoxicity test were negative. Immunosuppression was based on tacrolimus (Prograf, 2 mg/d; target trough levels of 9–13 μg/L for 90 days) and mycophenolate mofetyl (Cell Cept, Roche; 2 g/d) with induction using antithymocyte globulin (Thymoglobulin, Genzyme SAS; 0.5 and 1 mg/kg/d 2 days before transplantation, 1.5 mg/kg/d 3 days after transplantation) and etanercept (Enbrel, Pfizer; 50 mg at day 0 then 25 mg at days 3, 5, 10). Immediately after islet transplantation, C-peptide levels increased to 1.2 ng/mL and insulin requirements decreased slowly from 26 to 17 IU/d. Four days after transplantation, MPs were elevated slowly decreasing to 3.5 nmol/L PhtdSerEq at the end of the first month. One month after transplantation, we observed a peak of postprandial glucose above 11 mmol/L with a C-peptide drop to 0.3 ng/mL. Searches for anti-HLA antibodies by the Luminex technique remained consistently negative for HLA class I and II. In addition, the crossmatch assay performed with sera remained negative during the graft loss. No islet autoantibodies were detected against GAD 65 or IA2. Tacrolimus trough levels were 11.5 μg/L. There was no clinical or biological sign of infection. Polymerase chain reaction for cytomegalovirus (CMV) and Epstein-Barr virus (EBV) was undetectable. Peripheral lymphocyte count was 588/mm^3 with a CD4/CD8 ratio of 123/100. In the absence of an identified cause for islet loss, we hypothesized an acute cellular islet rejection episode and treated with steroid boluses (methylprednisolone, Pfizer, 10 mg/kg for 3 days then 6, 4, and 2 mg/kg for 1 day). Continuous intravenous insulin therapy (Umuline Rapid®, Lilly, 4 to 5 IU/h) prevented hyperglycemia. Four days after steroid therapy, C-peptide levels increased to 1.3 ng/mL, with insulin needs decreasing to 20 IU/d. Circulating MP levels increased slowly