Tissue Factor Produced by the Endocrine Cells of the Islets of Langerhans Is Associated With a Negative Outcome of Clinical Islet Transplantation

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There are strong indications that only a small fraction of grafts successfully engraft in clinical islet transplantation. One explanation may be the instant blood-mediated inflammatory reaction (IBMIR) elicited by tissue factor, which is produced by the endocrine cells. In the present study, we show that islets intended for islet transplantation produce tissue factor in both the transmembrane and the alternatively spliced form and that the membrane-bound form is released as microparticles often associated with both insulin and glucagon granules. A low–molecular mass factor VIIa (FVIIa) inhibitor that indirectly blocks both forms of tissue factor was shown in vitro to be a promising drug to eliminate the IBMIR. Thrombin-antithrombin complex (TAT) and FVIIa-antithrombin complex (FVIIa-AT) were measured in nine patients who together received 20 infusions of isolated human islets. Both the TAT and FVIIa-AT complexes increased rapidly within 15–60 min after infusion. When the initial TAT and FVIIa-AT levels were plotted against the increase in C-peptide concentration after 7 days, patients with an initially strong IBMIR showed no significant increase in insulin synthesis after 7 days. In conclusion, tissue factor present in both the islets and the culture medium and elicits IBMIR, which affects the function of the transplanted islets. *Diabetes* 54:1755–1762, 2005

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FVIIa, factor VIIa, FVIIa-AT, FVIIa-antithrombin complex; IBMIR, instant blood-mediated inflammatory reaction; mAbs, monoclonal antibodies; TAT, thrombin-antithrombin complex.

Today, the transplantation of isolated islets of Langerhans is a cell transplantation procedure nearing clinical acceptance as an effective treatment for type 1 diabetes. During 3 decades of trials, the difference in effectiveness between whole-pancreas transplantation and islet transplantation was puzzling, since insulin independence was achieved in only 10–15% of the recipients treated with islet transplantation, compared with 80% of those transplanted with whole pancreata.

A recently introduced novel protocol that combines previously known concepts has improved the results, and today clinical islet transplantation regularly leads to insulin independence (1). However, pancreatic islets from more than one donor are needed to reach insulin independence, and an evaluation of transplanted patients has shown that the functional capacity of the transplanted islets corresponds to only ~20% of that found in a nondiabetic person, despite the transplantation of islets from multiple donors (2). Together, these reports demonstrate that only a small fraction of the transplanted islets successfully engraft.

One explanation for this low level of engraftment may be the thrombotic/inflammatory reaction that we have recently characterized (3,4). It is elicited when islets come in direct contact with ABO-compatible blood and is characterized by activation and rapid binding of platelets to the islet surface, together with activation of the coagulation and complement systems. Within 15 min, leukocytes are found infiltrating the islets. After an hour, most of the islets are infiltrated by numerous leukocytes (both monocytes and granulocytes) resulting in disruption of islet integrity and islet loss. We call this thrombotic/inflammatory response the instant blood-mediated inflammatory reaction (IBMIR). In vivo evidence that the IBMIR occurs during islet infusion into the portal vein has been obtained in both a porcine transplantation model and in humans during clinical islet transplantation (3,4).

The IBMIR is triggered when tissue factor produced by the endocrine cells of the pancreatic islets elicits a clotting reaction. The content of tissue factor in the islets correlates with the strength of the IBMIR (5). The IBMIR can be totally blocked in vitro by inhibiting tissue factor with specific monoclonal antibodies (mAbs) or active site-

TABLE 1
Patients transplanted with human islets after kidney transplantation

	1	2	3	4	5	6	7	8	9	Mean ± SD
Age (years)	46	49	50	45	54	49	45	49	35	47 ± 5
Sex (F/M)	F	M	M	M	M	F	F	M	M	3/6
Blood group (A, B, O)	A	B	A	A	0	A	0	A	A	6, 1, 2
Body mass (kg)	72	71	78	89	88	67	57	63	63	72 ± 11
BMI (kg/m^2)	25	23	24	29	28	23	21	18	19	23 ± 3
Diabetes duration (years)	40	33	23	41	40	32	32	31	25	33 ± 6
Insulin requirement before tx (IU)	50	38	37	54	56	20	48	35	85	47 ± 17
Insulin/kg before tx (IU/kg)	0.69	0.53	0.48	0.61	0.61	0.3	0.84	0.56	1.35	0.66 ± 0.28
No. of islet infusions/patient	4	2	2	3	1	2	3	2	1	2.2 ± 0,9
Total no. of islet donors	4	3	2	5	2	3	3	3	1	2.9 ± 1.2
No. of islets per infusion (×1,000 IEQ)	179–250	250–260	250–400	315–750	780	230–550	400–500	200–320	300	407 ± 46
Islet purity (%)	70–80	70–75	70–80	70	70	70	70–97	70	75	73 ± 6

IEQ, insulin equivalents; tx, transplantation.

inactivated factor VIIa (FVIIa) (3). We (6) and others (7,8) have demonstrated that isolated human islets express at least 50 inflammation-associated genes, including those for monocyte chemotactic protein-1, interleukin-8, vascular endothelial growth factor, and macrophage inhibitory factor, suggesting that the tissue factor expression is part of a general inflammatory response in the isolated islets. Tissue factor and monocyte chemotactic protein-1 expression vary substantially from one islet preparation to another, on both the mRNA and protein levels, and can be regulated by ACE inhibitors and antioxidants such as nicotinamide (6). These observations indicate that its expression is induced in a manner analogous to that of other inflammation-related genes in transplanted tissue.

A likely explanation for the upregulation of these genes is the stress imposed on the islets from the time that the donor is admitted to the intensive care unit until engraftment of the isolated islets is achieved in the recipient. For the islets, the main stress factors are cortisol-induced peripheral insulin resistance and the direct effects of stress-related hormones on the donor before organ procurement, as well as prolonged periods of hypoxia during transportation, isolation, purification, and culture of the islets.

In the present study, we have further characterized tissue factor from cultured islets of Langerhans and demonstrated that the magnitude of the IBMIR during clinical islet transplantation affects islet function during the 1st week in vivo. We also attempted to block the tissue factor pathway by targeting the proteolytic activity of FVIIa using a soluble low–molecular mass inhibitor.

RESEARCH DESIGN AND METHODS

Islet isolation. Islets from human cadaver donors were isolated (according to a protocol approved by the ethical committee) as previously described (9–11) using a Liberase perfusion followed by continuous-density Ficoll gradient purification in a refrigerated COBE 2991 centrifuge (COBE Blood Component Technology, Lakewood, CO). Islet preparations were maintained in culture medium (CMRL 1066; ICN Biomedicals, Costa Mesa, CA) supplemented with HEPES, l-glutamine, gentamicin, fungizone (Gibco, Invitrogen, Paisley, Scotland, U.K.), and ciprofloxacin (Bayer Healthcare, Leverkusen, Germany) at 37°C (5% CO_2) for 1–4 days. The volume and purity were determined by microscopic sizing after staining with diphenylthiocarbazone. Viability was assessed as insulin secretion in response to a glucose challenge in a dynamic perfusion system (in 1.67, 16.7, and then 1.67 mmol/l glucose).

For PCR experiments, islets were collected on day 1 or 2 after isolation. Islets were either directly frozen or cultured until day 3 in medium containing 5.5 mmol/l glucose. The experiment was terminated by freezing the islets at −70°C.

Tubing loops as a model. The tubing loop experiments were performed as previously described (4,12). In summary, loops made of PVC (inner diameter, 6.3 mm; length, 380 mm) whose inner surface was furnished with immobilized heparin were used. A rocking apparatus, placed in a 37°C incubator, was used to generate blood flow inside the loops. Seven 60-min islet experiments were performed, with islets isolated from seven different donors. Ro69 (molecular mass = 391 g/mol; Hoffman-La Roche, Basel, Switzerland) a selective FVIIa inhibitor, was tested at 0, 10, 20, and 60 μmol/l. For each experiment, two loops of fresh human blood without islets, one containing CMRL 1066 (GibcoRBL, Grand Island, NY) and one containing 60 μmol/l Ro69, were included as controls. Fresh nonanticoagulated ABO-compatible human blood from the same donor (7 ml) was added to each loop. The loops were placed on the rocking device for a 5-min preincubation with Ro69. Thereafter, the loops were opened, and 100 μl CMRL 1066, with or without 5 μl of islets (~5,000 islet equivalents), was added to the loops and followed by another 60-min incubation on the rocking device at 37°C. Blood glucose levels were measured with a glucometer (Glucometer Elite; Bayer Diagnostics, Leverkusen, Germany) before the perfusion.

The blood was collected in 10 mmol/l EDTA (final concentration) and used for hematologic analysis (platelets, lymphocytes, monocytes, and granulocytes) and assays of coagulation activation (thrombin-antithrombin complex [TAT] and FVIIa-antithrombin complex [FVIIa-AT]). The blood samples were centrifuged at 4°C at 3,290*g* for 15 min, and the plasma was collected and stored at −70°C until analyzed.

Clinical islet transplantation. A modified Edmonton protocol was used. Major inclusion criteria for the study were long-standing type 1 diabetes, frequent uncontrollable hypoglycemic attacks and unawareness of these problems, and a previous transplantation with a cadaveric kidney graft due to end-stage renal disease. Nine consecutive patients were transplanted 20 times intraportally with human islets from a total of 26 donors (Table 1). Patients were already on immunosuppression because of a previous kidney graft. At the time of islet transplantation, immunosuppression was switched to the steriod-free protocol applied in Edmonton, including daclizumab, sirolimus, and tacrolimus (1). Blood samples were retrieved from a central vein catheter for the 1st h after transplantation and thereafter from a peripheral vein.

Affinity-purified anti-CEWGRAGRRTPH peptide antibodies. Antibodies were raised against CEWGRAGRRTPH conjugated to KLH. This peptide is specific for alternatively spliced tissue factor and comprises the last 11 COOH-terminal amino acids of the molecule (13). The peptide antibodies were thereafter affinity purified using a CEWGRAGRRTPH-sepharose column. The whole procedure was performed by Innovagen (Lund, Sweden).

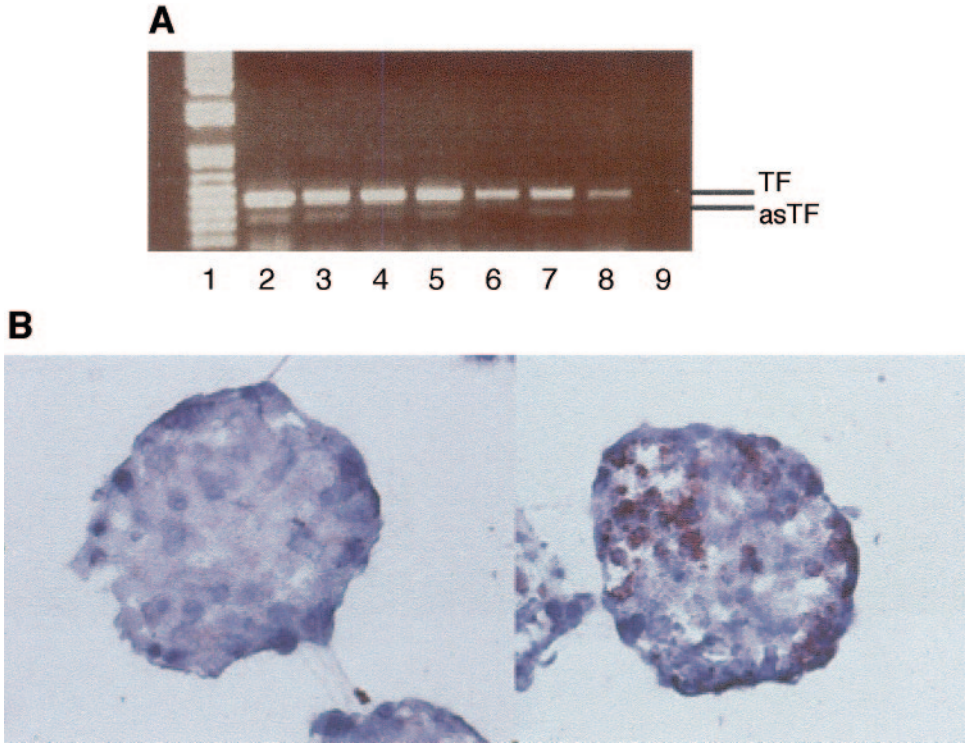

FIG. 1. Expression of the alternatively spliced form of tissue factor in human islets. *A*: Islets from six different donors were analyzed by RT-PCR. *Lane 1*, the molecular weight ladder; *lanes 2–7*, islets from the six different donors; *lane 8*, the positive control (placenta); *lane 9*, the negative control (without tissue). *B*: Sections of isolated islets were stained for alternatively spliced tissue factor using affinity-purified polyclonal anti–alternatively spliced tissue factor antibody. *Left panel*: Islet with nonspecific antibody. *Right panel*: Islet with specific anti–alternatively spliced tissue factor primary antibody present.

Immunohistochemical staining. Isolated human pancreatic islets were embedded in Tissue-Tek (Miles, Elkhart, IN) and snap frozen in liquid nitrogen. The samples were sectioned and stained with a rabbit anti-CEWGRAGRRTPH peptide antibody (13) or with an equal amount of nonspecific rabbit IgG (Dako, Glostrup, Denmark), followed by horseradish peroxidase–conjugated swine anti-rabbit Ig (Dako), respectively, using the peroxidase-antiperoxidase method.

Electron microscopy. Culture medium containing 10 mmol/l EDTA from day 2 or 3 after islet isolation was mixed with polyethylene glycol 6000 at a final concentration of 3% (wt/vol) at 4°C overnight. The supernatant was centrifuged at 2,756*g* at +4°C for 50 min, and the pellet was dissolved in PBS. This enriched preparation was then subjected to ultracentrifugation at 100,000*g* for 30 min.

The pellets were processed for ultrastructural immunocytochemistry by the low-temperature method (14) to preserve the antigenicity. Ultrathin sections placed on nickel grids were immunolabeled by the immunogold technique (15). Primary antibodies directed to tissue factor (mouse anti-human tissue factor 4509; American Diagnostica, Stamford, CT), insulin (guinea pig anti-insulin, Lot 0020D; Dako Cytomation, Dako), and glucagon (rabbit antihuman glucagon, Lot 0101B; Dako Cytomation) were labeled with corresponding gold-conjugated secondary antibodies (goat anti-mouse/5, 10, or 15 nm colloidal gold, goat anti–guinea pig/5 nm colloidal gold, and goat anti-rabbit/15 nm colloidal gold; Amersham International, Amersham, U.K.). Single and double labeling were performed. Sections were contrasted in uranyl acetate and lead citrate before analyzing in a Philips 201 electron microscope. Negative controls were included in each labeling experiment; one control in which the primary antibody was omitted and one where the primary antibody was replaced with isotype-matched nonspecific antibodies.

Blood and plasma analysis. Plasma FVIIa-AT complexes were quantified using sheep anti-human FVII (Affinity Biological, Ancaster, ON, Canada) as capture antibody and a biotinylated rabbit anti-human antithrombin as detecting antibody (Dako), followed by horseradish peroxidase–conjugated streptavidin (Pharmacia Amersham, Uppsala, Sweden). To measure TAT complexes, a commercially available pair of mAbs specific for thrombin and antithrombin (horseradish peroxidase conjugated) (Enzyme Research Laboratories, South Bend, IN) was used. A more detailed description of the two assays will be presented in an article in preparation. D-dimer was analyzed using the Imuclone D-dimer enzyme-linked immunosorbent assay kit from American Diagnostica, and C3a was analyzed using a previously described enzyme-linked immunosorbent assay (16). C-peptide in samples from fasting patients was assessed using an enzyme immunosorbent assay kit from Mercodia (Uppsala, Sweden).

The TAT and the FVIIa-AT complex levels, 15 min after the islet infusion, were correlated with the increase in fasting C-peptide values obtained 1 week after the islet infusion. The increase was calculated by subtracting the C-peptide level after 1 week with the C-peptide value obtained the day before islet infusion. The increase in fasting C-peptide for each islet infusion was used, i.e., one patient may generate more than one data point.

RT-PCR analysis. The organs used in isolation of islets for PCR analysis were obtained from three male and three female normoglycemic donors (aged 45 ± 6 years, BMI 25.9 ± 1.7 kg/m^2). Cytoplasmic RNA from islets was isolated as described by Gough (17). The primers were designed to extend over exons 4–6 to include both the transmembrane and alternatively spliced tissue factor, giving fragments of 408 and 248 bp, respectively, according to Bogdanov et al. (13). The sequence of the forward primer was 5′-ACTCCCCAGAGTTCACAC CTTACC (24 bp), and the sequence of the reverse primer was 5′-TGACCA CAAATAGGACAGCTCC (22 bp). RT-PCR was carried out in a total volume of 25 μl containing 5 μl cDNA sample, 800 nmol/l of each primer, 3 μmol/l $MgCl_2$, 0.2 mmol/l dNTPs, 0.025 units AmpliTaq Gold (Applied Biosystems, Foster City, CA), 2.5 μl buffer A (Applied Biosystems), and 9.875 μl water. The polymerase was activated at 94°C for 8 min, and amplification was done in 35 cycles of switching between 94°C for 30 s and 54°C for 30 s. The products were analyzed on 3% agarose gels with 0.5 μg/ml ethidium bromide.

Statistical analysis. All results were expressed as means ± SE. TAT, C-peptide, and FVIIa-AT levels for different time points were compared using Friedman ANOVA (Analyze-It, version 1.44; Analyze-It Software, Leeds, U.K.). The significance was determined at $\alpha = 0.05$.

RESULTS

Evidence of alternatively spliced tissue factor in cultured human islets. Human pancreatic islets have previously been shown to produce tissue factor on both the RNA and protein levels (3). To investigate whether human islets also produce the newly described alternatively spliced form, we analyzed 100 islets from six different individuals by RT-PCR using primers spanning both the transmembrane form and the soluble alternatively spliced form. We found that all the examined islet preparations produced mRNA associated with both forms of tissue factor (Fig. 1*A*). The transmembrane form was predominant, but significant amounts of the alternatively spliced form were also found.

Immunohistochemical analysis using the same affinity purified anti–alternatively spliced tissue factor antibody

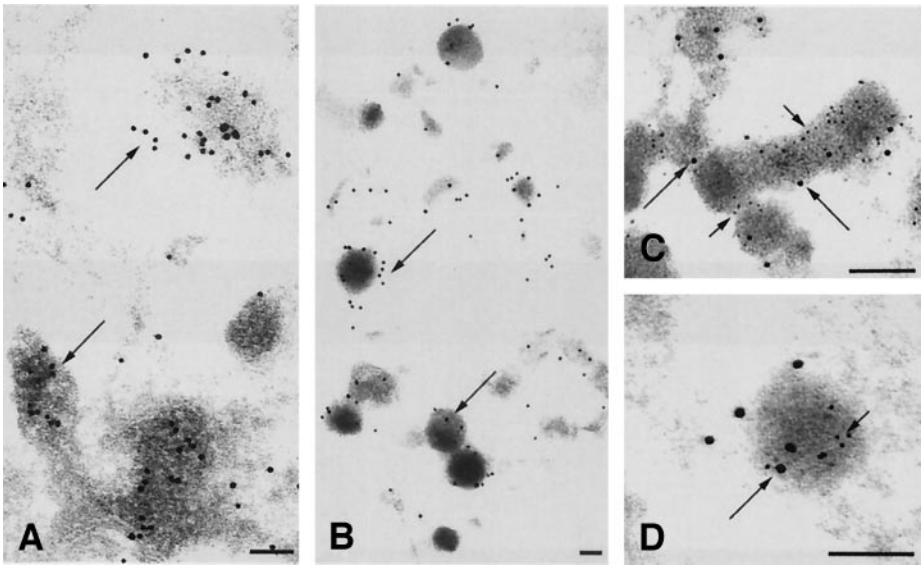

FIG. 2. Ultrastructural demonstration of tissue factor in granular remnants and in extracellular microparticles from isolated human pancreatic islets. Supernatants from human islets cultured for 2 days were analyzed by immunoelectron microscopy. *A*: Colloidial gold particles (15 nm) demonstrate the presence of tissue factor in extracellular granular remnants (→) reminiscent of the insulin granular core. Bar = 100 nm; ×70,000. *B*: Binding of anti–tissue factor antibodies visualized with 15-nm colloidal gold markers is obvious in more-or-less broken glucagon granules and in nonidentifiable electron-dense material (→). Bar = 100 nm; ×36,000. *C*: Microparticles with a morphology similar to the core of the insulin granules demonstrate a specifically colocalized immunoreactivity to anti–tissue factor (10 nm gold; long arrow) and anti-insulin (5 nm gold; short arrow) antibodies. Bar = 100 nm; ×110,000. *D*: Microparticles from the same experiment as in *C*, but with spherical particles with an electron density comparable to glucagon granules, revealed a specific immunoreactivity to anti-glucagon antibodies labeled with 15-nm gold markers (long arrow) and anti–tissue factor antibodies labeled with 5-nm gold markers (short arrow). Bar = 100 nm; ×154,000.

showed a distinctive staining of 10–20% of the cells in 80–90% of the pancreatic islets (Fig. 1*B*).

Electron microscopy of tissue factor from cultured human islets. Human islets were cultured for 2 days in 5.5 mmol/l glucose, and the released membrane-bound tissue factor found in the culture supernatants was analyzed by electron microscopy after concentration with polyethylene glycol and ultracentrifugation. The preparations were optimized for immunohistochemical staining and therefore did not yield optimal images for morphologic analysis.

Figure 2 shows that membrane-bound tissue factor was consistently associated with electron-dense microparticles. Both microparticles of unidentified origin and intact and destroyed glucagon- and insulin-containing granules could be identified. The association of tissue factor with glucagon- and insulin-containing granules was confirmed by double staining using a combination of anti–tissue factor/anti-glucagon and anti–tissue factor/anti-insulin, respectively. All negative controls proved to be negative.

Monitoring TAT, FVIIa-AT, and C-peptide levels during clinical islet transplantation. Nine patients underwent clinical islet transplantation after kidney transplantation, according to a modified Edmonton protocol as described in RESEARCH DESIGN AND METHODS. Altogether, the patients received 20 transplantations with organs from 26 multiorgan donors. Baseline samples were drawn before islet infusion, and additional samples were obtained at 15 and 60 min and 1, 3, and 7 days after islet infusion. TAT levels increased immediately after the islet infusion was stopped, and the peak level in the series was reached after only 60 min ($P = 0.0116$) (Fig. 3*A*). After 1 day, the levels had returned to baseline values. The generation of FVIIa-AT complexes also peaked at 60 min ($P = 0.016$) after the infusion, but the response was slightly delayed when compared with that of TAT (Fig. 3*B*). After 1 day, the levels had returned to baseline. D-dimer reflecting fibrinolysis reached maximum levels 1 day after the islet infusions (Fig. 3*C*). C-peptide was released immediately after infusion of the islets into the portal vein (Fig. 3*D*). The levels peaked 1 day after infusion ($P = 0.0005$) and declined thereafter, but never reached baseline levels, indicating that the endocrine cells of the islets produced C-peptide after 1 week ($P = 0.02$). C3a levels, reflecting complement activation, and platelet counts were also assessed but did not change after the islet infusions.

Correlation between TAT and FVIIa-AT levels and C-peptide generation. To investigate whether there is a possible link between the initial clotting reaction and fasting C-peptide production 7 days after transplantation, TAT generation the first 15 min after infusion of islets into the portal vein was plotted against the change in C-peptide concentration during the first 7 days after transplantation (Fig. 4*A*). A negative correlation was found, in which high TAT values never coincided with high C-peptide levels (and vice versa). A similar plot was obtained when FVIIa-AT values at 60 min were plotted against the change in C-peptide over 7 days (Fig. 4*B*).

Dose-dependent inhibition of IBMIR by the low–molecular mass FVIIa inhibitor Ro69 in the tubing loop model. In tubing loops containing human islets, a 60-min perfusion without Ro69 resulted in a marked consumption of platelets and leukocytes, combined with a pronounced activation of the coagulation system. Nearly all the platelets were consumed after 60 min in tubing loops without Ro69 (Fig. 5*A*). Granulocytes and monocytes were also partially consumed after 60 min, while lymphocytes were essentially unaffected (not shown). In addition, in the absence of Ro69, macroscopic clotting was seen and was accompanied by a significant

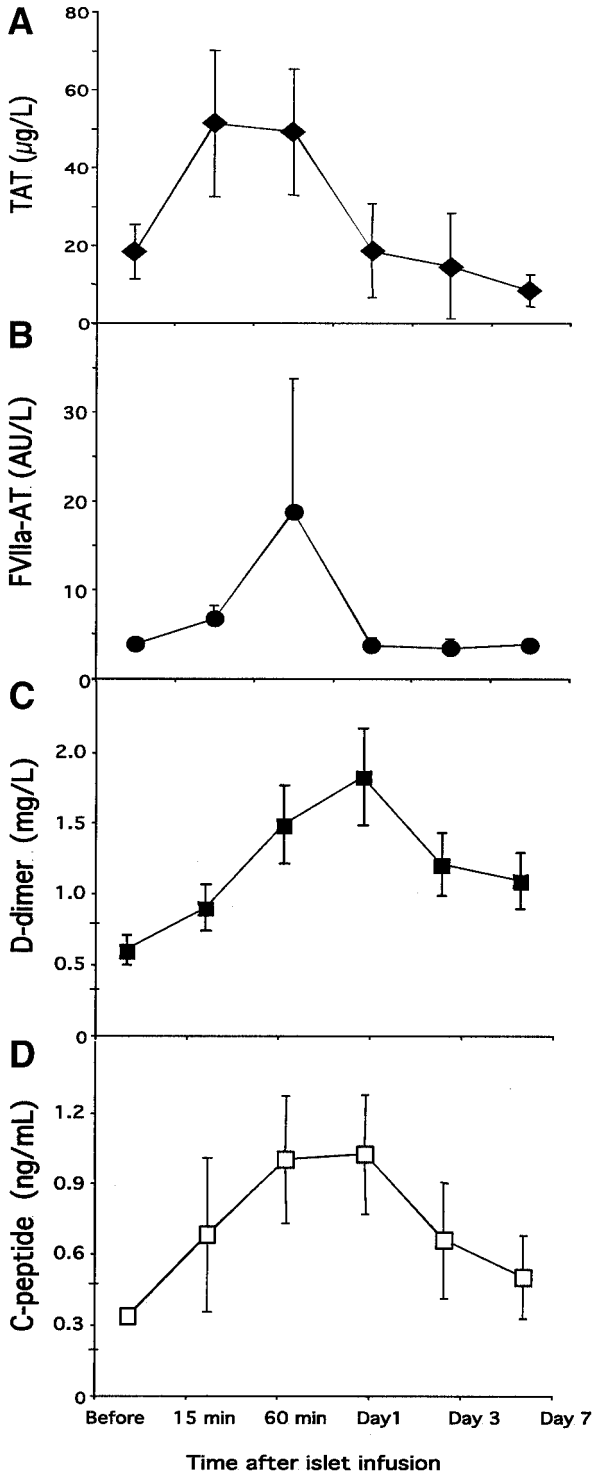

FIG. 3. Alterations in the concentration of TAT, FVIIa-AT, D-dimer, and C-peptide during clinical islet transplantation. Nine patients were transplanted with a total of 20 islet infusions, and the levels of TAT (*A*), FVIIa-AT (*B*), D-dimer (*C*), and C-peptide (*D*) were monitored. The 0-min point represents the level immediately before infusion of the islets and the 15-min point the levels 15 min after the infusion of the islets.

rise in FVIIa-AT and TAT complex levels (Figs. 5*B* and *C*, respectively).

Ro69 diminished cell consumption and cascade system activation in a dose-dependent manner. A beneficial effect on most parameters was observed at Ro69 concentrations

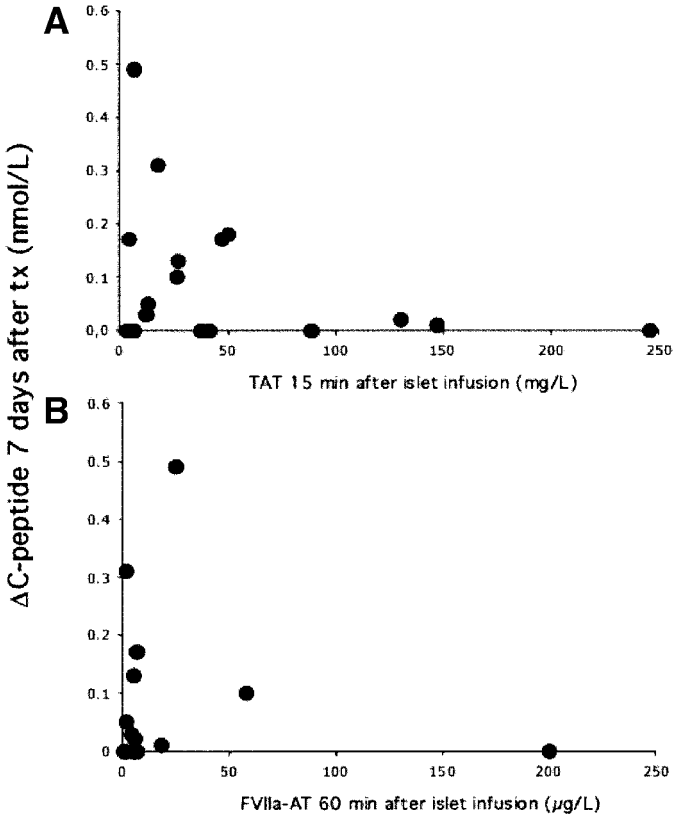

FIG. 4. Correlation between immediate TAT and FVIIa-AT generation and C-peptide production 1 week after clinical islet transplantation. *A*: Correlation between TAT levels at 15 min after islet infusion and the increase in fasting C-peptide concentration during the 1st week after transplantation. *B*: Correlation between FVIIa-AT levels at 60 min after islet infusion and the increase in fasting C-peptide concentration during the 1st week after transplantation.

as low as 10 μmol/l. The cell counts were fully restored at 60 μmol/l (Fig. 5*A*). The coagulation parameters FVIIa-AT and TAT decreased to nearly the same level as the medium control at 60 μmol/l Ro69 (Figs. 5*B* and *C*).

DISCUSSION

Tissue factor was first described as a transmembrane molecule belonging to the cytokine receptor superfamily type I. Recently, an alternatively spliced form of tissue factor was described that contains a unique 40–amino acid residue COOH-terminal sequence and in which exon 5 is deleted, resulting in a missing transmembrane motif (13). This soluble form of tissue factor was found to act as a procoagulant in the presence of phospholipids and to be expressed by various tissues. In particular, the lung and pancreas seemed to express significant amounts of this form. In our previous research, in which the endocrine cells were found to produce tissue factor, only the endocrine tissue and the vessel walls and not the exocrine part of the pancreas were positively stained for tissue factor with a mAb specific for tissue factor. In the present research, we have shown that the as–tissue factor form is expressed by the islets. It is therefore conceivable that the as–tissue factor found in whole-pancreas specimens is also mainly produced by the endocrine cells of the islets of Langerhans, cells that constitute ~1% of the tissue of the gland.

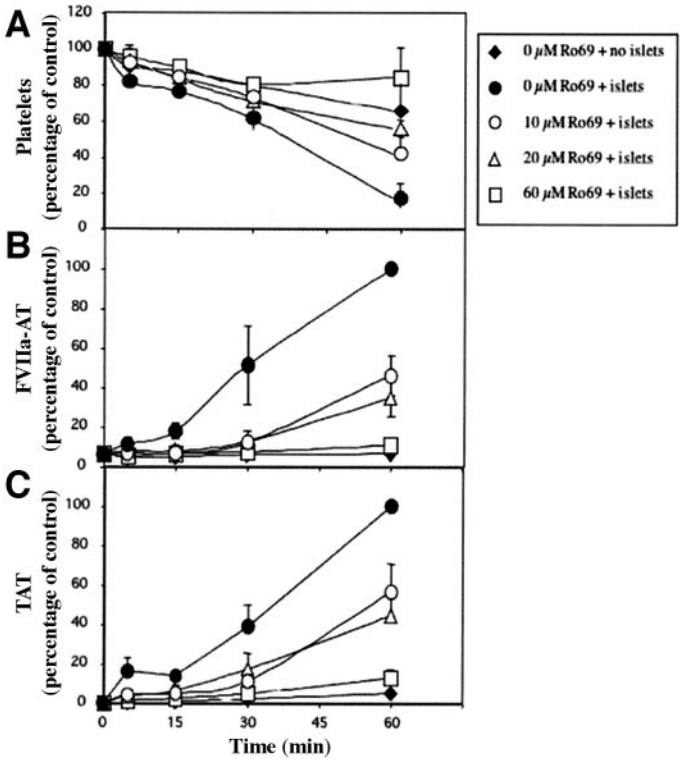

FIG. 5. The effect of Ro69 on the IBMIR induced when islets are incubated with ABO-compatible blood. Human islets were incubated in the tubing loop model filled with ABO-compatible blood containing Ro69 at different concentrations: ●, 0 mmol/l; ○, 10 mmol/l; △, 20 mmol/l; □, 60 mmol/l in platelets (*A*), FVIIa-AT (*B*), and TAT (*C*). The values are expressed as percentage of the values for the untreated control without islets at 0 min (platelets) or the untreated control containing human islets at 60 min (FVIIa-AT and TAT). ♦, blood without islets and Ro69.

Supernatants of cultured islets contain procoagulant activity, and previous studies in our laboratory have shown that all of this activity is precipitated by centrifugation at 100,000*g* and has been assumed to be associated with microparticles and cell debris (3). The activity can be abrogated by anti–tissue factor mAbs directed against the active site. Here, electron microscopy showed that tissue factor in pellets from ultracentrifuged culture medium was associated with electron-dense material such as unidentified microparticles and granules and granule debris of both α- and β-cell origin. Given our previous data indicating that tissue factor is present in the endoplasmatic reticulum, the Golgi complex, and the cytoplasmatic granules of the α- and β-cells, it appears that tissue factor can be secreted with glucagon and insulin into the supernatant.

Thus, both the isolated islets and cell debris from the islet cultures apparently contain tissue factor. This is the material that is infused into the portal vein of the patients and is assumed to trigger a detrimental IBMIR during clinical islet transplantation (3,4). In the present study, TAT levels, which reflect the magnitude of the IBMIR, were followed in nine patients receiving a total of 20 transplantations. TAT peaked soon after the islet infusion was terminated. The maximum concentrations were demonstrated in the 15- to 60-min interval and exceeded 50 μg/l, a level comparable to that obtained during sepsis (18). These data are consistent with our previously reported preliminary results (3). Also, FVIIa-AT complexes were generated soon after infusion. These complexes peaked after 60 min, underscoring the involvement of the tissue factor pathway in the IBMIR. In vitro inhibition of FVIIa by antithrombin has been described in a number of reports, but to our knowledge this is the first time that FVIIa-AT complexes have been demonstrated in vivo (19–21). The generation of D-dimer further strengthens the appearance of the IBMIR after islet infusion. Unlike the situation in the in vitro loop studies, a drop in platelet count and generation of C3a were not detectable. One reason why complement activation products generated by the IBMIR are not detected in vivo may be that they are eliminated by complement receptor–expressing cells, e.g., endothelial cells not available in the loop system. Furthermore, an increase in activation products and a consumption of platelets are obscured by the dilution resulting from blood passing through the liver. Supporting this is that the levels of TAT are ~100-fold less in vivo.

Even if it is likely to occur, a crucial question is whether the IBMIR has any impact on the outcome of clinical islet transplantation. Our transplanted patients are treated with insulin in the initial weeks after transplantation to let the transplanted islets rest. The fasting C-peptide levels 7 days after the transplantation will therefore reflect the basal production of endogenous insulin and can be used to mirror the function of the transplanted islets in the liver. The plot of the initial 15-min TAT values against the increase in the C-peptide values obtained 7 days after the transplantation demonstrated that high TAT levels were never combined with a high C-peptide production (and vice versa). This relationship suggested that an immediate, strong IBMIR was destructive to the production of insulin 1 week later. There are several possible explanations for this result. One is that the IBMIR has a direct destructive effect on the islets. Another is that large clot formation in the wide branches of the portal tree may prevent the islets from reaching the smaller vessels. This will cause the islets to succumb as a result of poor nutrition and hypoxia in the clot, and it is also most likely that the wide branches of the portal tree are not an ideal site for engraftment of the islets. There were, however, islets that triggered a low level of TAT generation but still produced no C-peptide. Here, primary nonfunction due to alternative mechanisms must be operative (e.g., apotosis) (22).

There are two strategies to block the IBMIR. Systemic treatment of the patients with anticoagulants, e.g., tissue factor pathway inhibitors, or pretreatment of the islets to make them less thrombogenic. Systemic inhibition of the tissue factor pathway at the FVIIa level is tempting because of the low blood concentration of factor VII and, in particular, its activated form, VIIa. Direct inhibition of tissue factor is much more complex in that there are no low molecular weight inhibitors available. Instead, recombinant proteins can be used, e.g., tissue factor pathway inhibitor and site-inactivated FVIIa (23). In particular, tissue factor pathway inhibitor has a complicated inhibition mechanism. A small–molecular mass inhibitor can be developed into an agent that can be given orally. In the context of clinical islet transplantation, this gives the opportunity to specifically maintain high levels of the drug in the portal vein system.

In the present study, we demonstrate that Ro69 is a potent inhibitor of the IBMIRs that occur when human islets come in contact with ABO-compatible blood. In this system, the inhibition of FVIIa by Ro69 inhibited macroscopic clotting and completely blocked the generation of FVIIa-AT and TAT. Administration of Ro69 in the loop model system also abrogated platelet consumption. The most striking effects of Ro69 were seen between 10 and 60 μmol/l, a range that is considerably higher than the concentrations intended to be used in patients. As discussed above, an in vitro system generates higher coagulation activation product levels than would be the case in the dynamic in vivo situation. Thus, inhibition of FVIIa in vivo will require much lower concentrations of an inhibitor. The concentration of Ro69 required to abrogate IBMIR in clinical islet transplantation must therefore be estimated from data in preclinical animal studies.

We have previously shown that islets from different donors vary in their content of tissue factor and that tissue factor expression is significantly lowered by culturing the islets in the antioxidant nicotinamide (5). A finding that may be related to this is that high-dose orally administered nicotinamide has favorable effects on the outcome of clinical islet transplantion using only one donor, with insulin independence being attained in ~15–20% of the patients (24). One possible implication of the findings reported here is that tissue factor expression should be quantified before transplantation. If the tissue factor levels are high, the islets could be further cultured under optimal conditions in nicotinamide until the tissue factor concentration has dropped to acceptable levels. To further reduce the tissue factor expression in vivo, the patients could be given oral nicotinamide treatment.

In whole-organ transplantation, the length of the cold ischemia time is an important parameter that determines the magnitude of the complement-driven reperfusion injury (25). This injury is triggered by ischemia-induced alterations in the plasma membranes of the endothelial and parenchymal cells of the transplanted organs and indicates a requirement for a functional vascular system in the transplant. IBMIR is the corresponding reaction in islet cell transplantation, in which tissue factor and other induced inflammatory genes trigger a destructive thrombotic/inflammatory reaction when the islets come in contact with interstitial fluids and, in particular, with whole blood (4). Even though the IBMIR has only been linked thus far to islet cell transplantation, it is possible that it also occurs when ischemic isolated cells from other organs are infused into whole blood (e.g., hepatocytes) and may therefore represent a more general problem in cell transplantation.

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