Rapid Publication

Incompatibility Between Human Blood and Isolated Islets of Langerhans

A Finding With Implications for Clinical Intraportal Islet Transplantation?

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The remarkable difference in success rates between clinical pancreas transplantation and islet transplantation is poorly understood. Despite the same histocompatibility barrier and similar immunosuppressive treatments in both transplantation procedures, human intraportal islet transplantation has a much inferior success rate than does vascularized pancreas transplantation. Thus far, little attention has been directed to the possibility that islets transplanted into the blood stream may elicit an injurious incompatibility reaction. We have tested this hypothesis in vitro with human islets and in vivo with porcine islets. Human islets were exposed to nonanticoagulated human ABO-compatible blood in surface-heparinized polyvinyl chloride tubing loops. Heparin and/or the soluble complement receptor 1 (sCR1) TP10 were tested as additives. Adult porcine islets were transplanted intraportally into pigs, and the liver was recovered after 60 min for immunohistochemical staining. Human islets induced a rapid consumption and activation of platelets. Neutrophils and monocytes were also consumed, and the coagulation and complement systems were activated. Upon histological examination, islets were found to be embedded in clots and infiltrated with CD11$^+$ leukocytes. Furthermore, the cellular morphology was disrupted. When heparin and sCR1 were added to the blood, these events were avoided. Porcine islets retrieved in liver biopsies after intraportal islet allotransplantation showed a morphology similar to that of human islets perifused in vitro. Thus, exposure of isolated islets of Langerhans to allogenic blood resulted in significant damage to the islets, a finding that could explain the unsatisfactory clinical results obtained with intraportal islet transplantation. Because administration of heparin in combination with a soluble complement receptor abrogated these events, such treatment would presumably improve the outcome of clinical islet transplantation by reducing both initial islet loss and subsequent specific immune responses.
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Type 1 diabetes affects ~10 million people worldwide, and the number of people with this disease is predicted to increase to as many as 25 million by 2010 (1). At present, the only way to achieve permanent normoglycemia in patients with type 1 diabetes is by renewal of the insulin-producing β-cells through the transplantation of either a vascularized pancreatic graft or isolated islets of Langerhans. Within a few days after vascularized pancreatic transplantation, patients almost invariably become insulin free, and ~60% of them remain insulin free 3 years after transplantation (2). After islet transplantation, the results are vastly inferior, with ~10% of patients becoming insulin independent and ~7% remaining insulin free after 1 year (3). The reason for this remarkable difference in success rates is obscure. The histocompatibility barrier, the underlying autoimmune disease, and the immunosuppressive agents used are the same in both types of transplantation. However, whereas the islets are left in the native pancreas during pancreas transplantation, islet transplantation usually entails the injection of the islets into the portal vein, thereby embolizing the islets into the liver. Although several other transplantation sites have been evaluated in experimental models, intraportal transplantation is the only site established for clinical islet transplantation.

In early attempts at islet transplantation, portal hypertension, hepatic infarction, and even patient death occurred and

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β-TG, β-thromboglobulin; BW, body weight; ELISA, enzyme-linked immunosorbent assay; FXIa-AT, factor XIa–antithrombin complex; FXIIa-AT, factor XIIa–antithrombin complex; HRP, horseradish peroxidase; MPO, myeloperoxidase; PFA, paraformaldehyde; PMN, polymorphonucleocyte; PVC, polyvinyl chloride; sCR-1, soluble complement receptor 1; TAT, thrombin-antithrombin complex.

were thought to be associated with exocrine contamination of the islet preparations (4,5). Today, islet preparations are much purer, and the incidence of portal hypertension, liver infarction, and patient death is much reduced but has not been abolished (6,7).

Several investigators have reported early graft nonfunction after intraportal islet transplantation in various animal models. In some models, the islets resumed function after days or weeks; in other models, the islets never regained function. Recently, some investigators ascribed this phenomenon to nonspecific inflammatory reactions, with macrophages and nitric oxide acting as mediators of the reactions (8–10).

Thus far, little or no attention has been focused on the possibility that the islets transplanted into the portal vein may be treated by the immune system as foreign particles, since collagen and other matrix molecules of the graft, normally hidden by the endothelial cells, are now exposed to the host blood. We postulate that the islets may be damaged or destroyed by mechanisms that are triggered by introducing the islets into the bloodstream.

To test this hypothesis, we developed an in vitro islet perifusion system that mimics the situation in vivo immediately after transplantation. In this system, isolated human islets were exposed to fresh nonheparinized ABO-compatible human blood in polyvinyl chloride (PVC) loops whose inner surface was covered with surface-bound heparin. It was then found that the islets elicited prompt binding and activation of platelets, binding of leukocytes, and activation of the coagulation and complement systems, resulting in disruption of the integrity of the islets. This chain of events could be prevented by the addition of heparin in combination with the complement inhibitor soluble complement receptor 1 (sCR-1). Furthermore, when adult porcine islets were transplanted intraportally into pigs and retrieved in biopsies after 60 min, they showed a morphology similar to that of the human islets exposed to human blood in the perifusion loops.

We conclude that the inflammatory events observed both in vitro and in vivo could provide an explanation for the unsatisfactory results seen with islet allotransplantation. If these events were counteracted, a larger islet mass could presumably engraft and escape subsequent specific immune responses, thereby improving the outcome in clinical islet transplantation.

RESEARCH DESIGN AND METHODS

Islet isolations. Islets were isolated from six human pancreases after consent was obtained from either the organ donor registry or living relatives. The organs were obtained from three male and three female normoglycemic donors (aged 31–58 years, four with blood group A and two with blood group B). The islet preparations were of good quality, but because the total islet volume was too low for clinical transplantation, they were available for use in the present study. Islets were isolated at the Giessen Islet Isolation and Transplant Center according to a modified semiautomated digestion-filtration method, followed by purification on a continuous density gradient in a refrigerated COBE 2991 centrifuge (COBE Blood Component Technology, Lakewood, CO) (11,12). The islet preparations were then placed in untreated culture flasks and maintained in suspension culture at 24°C (5% CO_2 in humidified air) for 4–7 days. The medium was changed twice during this period. The volume and purity of the islets were determined by microscopical sizing on a grid after staining with diphenylthiocarbazone, and viability was assessed by membrane integrity testing (trypan blue exclusion). Also, insulin content and secretion in response to a glucose challenge in a static incubation system (in 1.67 and 16.7 mmol/l glucose) were determined. In addition, islets from one preparation were transplanted into streptozotocin-diabetic nude mice (treated with 250 mg/kg i.v.; Sigma, St. Louis, MO).

Adult porcine islets were isolated from the pancreases of 2-year-old Swedish Landrace pigs at Huddinge Hospital according to a modified Ricordi method (11). The islets were cultured in culture flasks at 37°C in a gas phase consisting of 5% CO_2 in humidified air for 1–7 days. The culture medium was changed every second day. Quality, purity, and viability of the islets were assessed as described above for human islets.

Human islet perifusion with human blood in heparinized PVC tubing loops. Islets were perifused in a device modified from a state-of-the-art system used for testing biomaterials (13,14). The device consisted of loops made of PVC tubing (diameter = 6.3 mm, length = 400 mm) with immobilized heparin on the inner surface. A rocking apparatus (Heto Lab Equipment, Alleröd, Denmark), placed in an incubator at 37°C, was used to generate blood flow inside the loops (~100 ml/min). As many as seven devices could be rocked simultaneously. The heparin-coated components were kindly supplied by Corline Systems (Uppsala, Sweden).

Islets were harvested from the culture flasks and washed twice in RPMI 1640 (ICN Biomedicals, Costa Mesa, CA), and 5 µl of islets (~5,000 IEQ), suspended in 100 µl of RPMI 1640, were placed in the loops. Fresh human blood from healthy volunteers was collected in surface-heparinized 60-ml syringes with a cannula (18-guage, Microlance; Becton Dickinson, Franklin Lakes, NJ) that was connected to a surface-heparinized silicon tubing. During sampling, the syringe was rotated continuously. Blood (7–8 ml) was then added to each loop, leaving an air volume of ~4 ml. After they were filled, the loops were closed with a stainless-steel connector and placed on the rocking device in the incubator. The loops were rocked at an amplitude setting that prevented blood from making contact with the connectors. Every experiment included a control loop containing blood supplemented with 100 µl of RPMI 1640 but no islets. ABO-compatible human blood was obtained from nine healthy volunteers.

There were 14 islet perifusions of 60 min each that were performed with islets isolated from six different pancreases. Three sets of loop perifusions were also performed to determine the time course of the events occurring during the 60-min experiments. In these experiments, five loops were used simultaneously. One was a control loop with blood and RPMI 1640 only, while the other four contained islets. At 5, 15, 30, and 60 min, one of the loops containing islets was removed for sampling. Because only a certain number of loops could be rocked simultaneously, only one control loop was used for each experiment and removed for sampling at 60 min. In three separate experiments, however, the dynamics of the changes in the control loops were also studied. In these experiments, four control loops were rocked simultaneously, and loops were removed for sampling at 5, 15, 30, and 60 min, respectively.

Heparin and/or sCR-1 was added to the system in seven experiments. As before, five loops filled with blood from one donor were used simultaneously. One loop contained islets and blood without additives; one contained islets and blood supplemented with heparin (4 IE/ml blood; Heparin LEO; 5,000 units/ml; Löwen, Malmö, Sweden); one contained islets and blood supplemented with sCR-1 (TP10; 100 µg/ml blood; kindly supplied by Imutran/Novartis, Cambridge, U.K.); one contained islets and blood supplemented with heparin (4 IE/ml blood) and sCR-1 (100 µg/ml blood); and one was a control loop. All of the loops were rocked for 60 min.

Blood glucose levels were measured with a glucometer (Glucometer Elite; Bayer Diagnostics, Leverkusen, Germany) before perifusion. EDTA-treated blood and plasma collected before and after islet perifusion were used for hematologic analysis and assays of complement, coagulation, myeloperoxidase (MPO), β-thromboglobulin (β-TG), and insulin. After every perifusion, macroscopic blood clots were removed, and the remaining blood was filtered through 70-µm diameter filters (Filcons, Cuptype; DAKO, Glostrup, Denmark). Both macroscopic blood clots and material recovered on filters were frozen in liquid nitrogen for immunohistochemical staining. For immunohistochemical comparison, nonperifused islets were also frozen and stained in the same way that islets recovered after perifusion were.

Blood and plasma analysis. Platelet counts and differential leukocyte counts in EDTA-treated blood were obtained using a cell-particle counter (Technicon H3 RTX, Bayer Diagnostics). C3a was analyzed using a sandwich enzyme-linked immunosorbent assay (ELISA) as described by Nilsson et al. (15). For the analysis of sC5b-9, a modification of the method described by Mollnes et al. was used (15,16). Factor XIa–antithrombin complex (FXIa-AT) and factor XIIa–antithrombin complex (FXIIa-AT) were analyzed by ELISA as described by Sanchez et al. (17). Thrombin-antithrombin complex (TAT) was analyzed using Enzygnost (TAT micro; Behringwerke, Marburg, Germany). MPO and β-TG were quantified with commercial ELISA kits (BIOXYTECH MPO-EIA, Oxis International, Portland, OR, and ASSERACHROM B-TG, Diagnostica Stago, Asnières-sur-Seine, France). The plasma concentration of insulin was analyzed before and after islet perifusion with a commercial ELISA kit (DAKOPATT, Glostrup, Denmark).

Intraportal allotransplantation of adult porcine islets. Five 6- to 8-week-old Swedish Landrace piglets weighing 21.1–24.8 kg (23.3 ± 0.71) were anesthetized using tiletamin (50 mg/ml) and zolezepam (50 mg/ml) (Zoletil 100; Virbac Laboratories, Carros, France) given at 6 mg/kg body weight (BW), 2 mg/kg BW of Xylazine (Rompun Vet, 20 mg/ml; Bayer AG, Leverkusen, Germany), and 0.04 mg/kg BW atropine. Morphine (20 mg) was given intravenously as an analgesic. A tracheotomy was performed, and the pigs were ventilated with O_2/N_2

TABLE 1
Blood cell counts and coagulation and complement parameters before and after 60 min of human islet perifusion with fresh ABO-compatible human blood

	Before	Control	Human islets (no additives)	*P*
n	14	14	14	—
Platelets ($\times 10^9$)	233 ± 13.8	161.1 ± 9.3	5 ± 0.3	<0.001
Neutrophils ($\times 10^9$)	3.23 ± 0.33	3.03 ± 0.32	0.83 ± 0.18	<0.001
Monocytes ($\times 10^9$)	0.36 ± 0.03	0.36 ± 0.04	0.03 ± 0.01	<0.001
Lymphocytes ($\times 10^9$)	1.91 ± 0.12	1.77 ± 0.12	1.29 ± 0.12	<0.001
C3a (ng/ml)	84 ± 4.7	507 ± 115	1,259 ± 125.1	<0.001
C5b-9 (AU/ml)	15.6 ± 2.9	95 ± 30	213 ± 43.4	0.006
FXIIa-AT (μmol/l)	0.09 ± 0.01	0.36 ± 0.15	12.9 ± 0.9	<0.001
FXIa-AT (μmol/l)	0.06 ± 0.01	0.12 ± 0.03	4.74 ± 0.48	<0.001
TAT (μg/ml)	12.5 ± 5.2	316 ± 100	20,537 ± 1,973	<0.001
β-TG (IU/ml)	1,056 ± 225	2,704 ± 451	6,778 ± 299	<0.001
MPO (ng/ml)	15.1 ± 3.2	17.6 ± 4.6	21.5 ± 3.7	0.85
Insulin (pmol/l $\times 10^3$)	0.06 ± 0.01	0.06 ± 0.01	30.3 ± 4.7	<0.001

Data are *n*, means ± SE, or *P*. Control loops contained blood and medium, but no islets. The degree of significance is reported with respect to the controls. AU, arbitrary units.

(30%/70%). During the experiment, ketamine (Ketaminol vet; Veterenaria AG, Zurich, Switzerland) at 20 mg · kg^{-1} BW · h^{-1}, pancuronium bromide (Pavulon; Organon Teknika, Boxtel, Holland) at 0.25 mg · kg^{-1} BW · h^{-1}, and morphine at 0.5 mg · kg^{-1} BW · h^{-1} were administered by continuous intravenous infusion. In all five piglets, a catheter (1.2/32 mm, Venflon; Ohmeda AB, Helsingborg, Sweden) was placed in the superior mesenteric vein, and 3.193–19.575 IEQ/kg BW (7,523 ± 3,037) porcine islets suspended in 10 ml of RPMI 1640 were infused intraportally over 5 min. After 60 min, the pigs were killed by intravenous administration of 20 mmol potassium chloride, and the livers were removed.

Immunohistochemical staining. Macroscopic blood clots collected from the loops were mounted in embedding medium (Tissue-Tek; Miles, Eckhart, IN) and then snap-frozen in liquid nitrogen. Likewise, islets recovered on filters (after perifusion with blood supplemented with heparin) were embedded in tissue glue and snap-frozen. Islets were sectioned and stained with horseradish peroxidase (HRP)-conjugated mouse anti-human CD-41a (R&D Systems, Abingdon, U.K.), mouse anti-fibrin (Immunotech, Marseilles, France), and anti-CD11b (clone 2LPM 19c, DAKO). HRP staining with mouse anti-human IgM and IgG and rabbit anti-human IgA (DAKO), rabbit anti-human-C3c (DAKO), and mouse anti-human C5b-9 (clone MCaE11, DAKO) were also performed. C1q staining was performed using a rabbit anti-human C1q (DAKO).

After removal of the livers in the intraportal porcine allotransplantation experiments, the portal system was exposed, and blood clots and liver biopsy samples were fixed in paraformaldehyde (PFA) (Histofix; Histolab Products, Västra Frölunda, Sweden) or snap-frozen in liquid nitrogen. Sections of blood clots and liver biopsies were then stained with hematoxylin and eosin.

Statistical analysis. All results are expressed as means ± SE. Statistical significance for the islet loop perifusions without the addition of sCR-1 or heparin was calculated using paired Student's *t* tests. For islet loop perifusions performed with and without sCR-1 and/or heparin, analysis of variance with repeated measurements was performed (Fisher's exact test). Furthermore, Friedman's one-way analysis of variance was also performed on the data from the latter experiments, giving similar statistical significances (not shown).

RESULTS

Islet preparation. The purity of the human islet preparations was between 75 and 90% (81.7 ± 2.8%), and the viability was between 80 and 96.5% (90.3 ± 2.4%). The static insulin stimulation index (ratio of stimulated versus unstimulated insulin secretion) was 1.2–7.0 (3.9 ± 0.9), and the insulin content per islet equivalent (standard volume of an islet 150 μm in diameter) was 40.8–93.3 μU (66.3 ± 7.5). Islets from one preparation were transplanted into diabetic nude mice for in vivo quality testing, leading to subsequent normalization of blood glucose values within 24 h (data not shown). The porcine islets used for the in vivo intraportal allotransplantation experiments had a purity between 87 and 92% (89.7 ± 0.83%).

Blood and plasma analysis after human islet perifusion. In loops containing islets, there was marked consumption of platelets, neutrophils, and monocytes. Concurrently, there was a pronounced activation of the coagulation and complement systems and extensive clotting (Table 1). In the control loops, no clotting was seen, and the only apparent change after 60 min was a loss of ~30% of the platelets and a slight increase in β-TG.

In loops containing islets, almost all of the platelets were consumed during the first 5 min, and β-TG was concurrently secreted (Fig. 1*A*). The decrease in neutrophil and monocyte counts occurred slightly after and at a slower rate than the platelet counts decreased, whereas the lymphocyte counts were almost unaffected (Fig. 1*B*). Clotting in the tubing was evident after 5 min, and there was a concomitant increase in FXIIa-AT, FXIa-AT, and TAT with time (Fig. 1*C*). After ~5 min of perfusion, C3a began to increase (Fig. 1*C*), whereas the main increase in C5b-9 occurred after 30 min. During the dynamic control loop perifusions, only ~7% of the platelets were lost after 5 min, whereas 15% were lost after 15 min and 22% were lost after 60 min. The leukocytes remained mostly unaffected throughout the 60 min.

Addition of sCR-1 to the loops prevented complement activation but did not influence the drop in the hematologic cell counts, the clot formation, the generation of FXIIa-AT, FXIa-AT, and TAT, or the release of β-TG and insulin (Table 2). Addition of heparin to the blood reduced the cellular changes, prevented clotting, decreased complement activation, and significantly reduced FXIIa-AT, FXIa-AT, and TAT. Addition of heparin and sCR-1 in combination inhibited both coagulation and complement activation and prevented cellular consumption (Table 2). Indeed, the addition of heparin in combination with sCR1 resulted in most read-out values being approximately equal to or even lower than those of the control loops (Tables 1 and 2).

Immunohistochemical staining of human islets after loop perifusion. Islets perifused without additives were

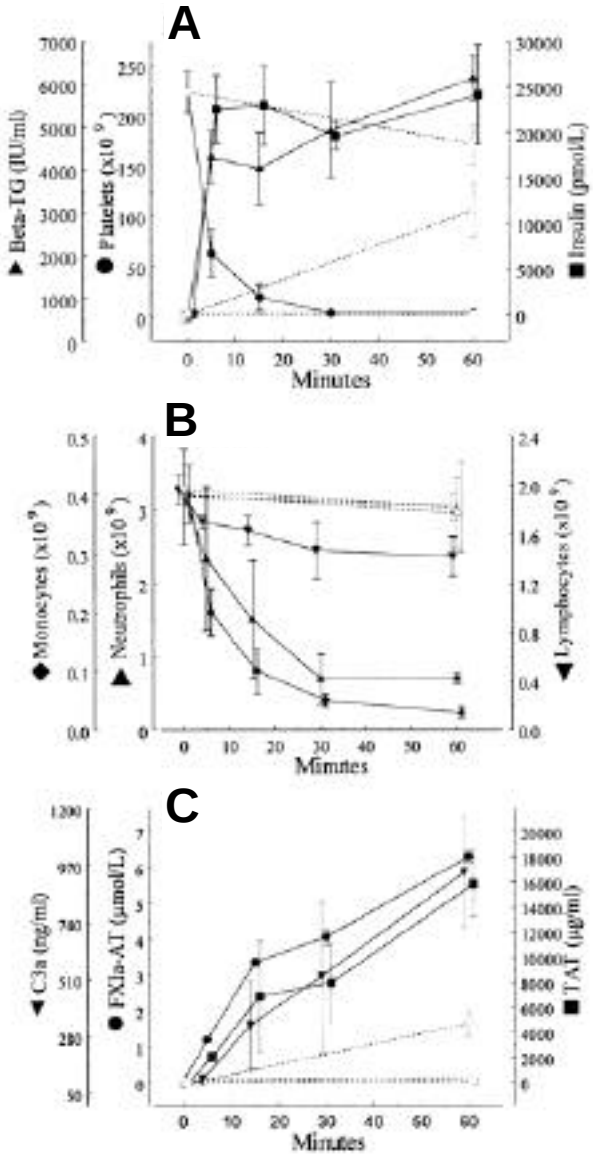

FIG. 1. *A*: **Changes in platelets (●), β-TG (▲), and insulin (■) during 60 min of human islet perifusion with unmodified human blood. Also illustrated are the values at 0 and 60 min (connected with a dotted line) in the control loops: platelets (○), β-TG (△), and insulin (□).** *B*: **Changes in neutrophils (▲), monocytes (◆), and lymphocytes (▼) during 60 min of human islet perifusion with unmodified human blood. Also illustrated are the values at 0 and 60 min (connected with a dotted line) in the control loops: neutrophils (△), monocytes (◇), and lymphocytes (▽).** *C*: **Changes in FXIa-AT (●), TAT (■), and C3a (▼) during 60 min of human islet perifusion with unmodified human blood. Also illustrated are the values at 0 and 60 min (connected with a dotted line) in the control loops: FXIa (○), TAT (□), and C3a (▽).**

invariably embedded in large clots. After 5 min, there was already extensive binding of platelets and fibrin to the islet surface, completely surrounding the islet (Fig. 2*B*). Platelets adhering to the islet surface at 5 min were strongly positive for P-selectin (Fig. 2*C*). By 5 min, the $CD11^+$ polymorphonucleocytes (PMNs) and monocytes were mainly observed in the fibrin clot surrounding the islets (Fig. 2*D*). By 60 min, almost all of the blood in the loops had clotted, embedding the islets (Fig. 2*E*). The number of $CD11^+$ PMNs and monocytes sequestered around the islets had also increased, and large numbers of $CD11^+$ leukocytes could be seen penetrating the islets (Fig. 2*F*). However, staining for C1q, C5b-9, hIgM, hIgG, and IgA was negative. Although most of the islets were also free of C3c, this cleavage product was sometimes found in the vicinity of $CD11^+$ cells, close to the islets. The morphology of the islets was disrupted, with condensed pyknotic islet cell nuclei (Fig. 2*B–F*). Control islets not exposed to blood did not stain positive for any of the monoclonal antibodies used.

Findings similar to those just described were obtained when sCR-1 was added to the blood, except that no deposition of C3c could be detected. When heparin was added to the blood, the islets became embedded in microclots, rather than in macroscopic clots. The histology of these islets was very similar to that of islets perifused with blood without additives or islets perifused with sCR-1–treated blood, with extensive platelet and fibrin deposition, $CD11^+$ leukocyte infiltration, and disrupted islet morphology. However, islets perifused with blood supplemented with both sCR-1 and heparin had only a thin layer of platelets and fibrin adhering to their surfaces, and $CD11^+$ leukocyte infiltration was either minimal or absent (Fig. 2*G* and *H*). These islets also had a normal morphology, similar to that of the control islets that were not exposed to blood (Fig. 2*A* and *H*).

Insulin release during islet perifusion. The glucose concentration in the blood before the islet perifusion was 4.5–7.4 mmol/l. When the islets were exposed to blood, a large increase in plasma insulin concentration was observed, with the levels rising from 63 ± 20 to 22,513 ± 3,766 pmol/l during the first 5 min (Fig. 1*A*). Addition of sCR-1, heparin, or both heparin and sCR-1 did not significantly affect the insulin concentration in the plasma at 60 min.

Histological evaluation of the liver after intraportal islet transplantation in pigs. In the portal veins, blood clots were found in all of the livers. The thrombi were often partially adherent to the endothelium and branched out into the portal tree (Fig. 3*A*). Histological examination revealed frequent portal vein thrombi, occasionally with entrapped islets. The entrapped islets showed a disrupted morphology (Fig. 3*B–D*). A fibrin tail could sometimes be observed extending away from the islets (Fig. 3*B*).

DISCUSSION

The reason for the poor results that have been obtained with clinical islet transplantation has remained an enigma for the past 20 years. In the present study, in which human islets were exposed to human blood in an in vitro model, a far-reaching series of inflammatory events was found to occur. The most striking event was the occurrence of macroscopic coagulation that occurred within 5 min. There was concomitant binding of platelets to the islet surface, rapid loss of platelets from the blood, and fibrin formation that generated a capsule surrounding the islets. Simultaneous secretion of β-TG and upregulation of P-selectin indicated platelet activation.

The platelet-binding ligand on the islet surface has not been identified; however, type I, III, IV, and V collagens are found within and surrounding human islets, and it is known that collagens mediate platelet binding and activation (18,19). The integrins $\alpha_2\beta_1$, CD36, P65, and GPVI have all been proposed as platelet collagen receptors; thus, the extracellular matrix proteins are likely to be ligands of platelet binding and activation when isolated islets are exposed to blood (20).

TABLE 2
Blood cell counts and coagulation and complement parameters before and after 60 min of human islet perifusion with ABO-compatible fresh human blood and blood supplemented with sCR1 and heparin

	Human islets					
	No additives	sCR1	Heparin	sCR1 + heparin	*F*	*P*
n	7	7	7	7	—	—
Platelets ($\times 10^9$)	5.2 ± 0.6	5 ± 0.6	114 ± 17	113 ± 20	14.5	0.004
Neutrophils ($\times 10^9$)	0.46 ± 0.14	0.66 ± 0.12	2.56 ± 0.43	2.68 ± 0.53	19.5	0.004
Monocytes ($\times 10^9$)	0.02 ± 0.004	0.02 ± 0.004	0.28 ± 0.06	0.28 ± 0.07	9.8	0.004
Lymphocytes ($\times 10^9$)	1.11 ± 0.23	0.93 ± 0.22	1.60 ± 0.20	1.62 ± 0.23	12.6	0.003
C3a (ng/ml)	1,424 ± 160	355 ± 64.6	565 ± 143.6	219 ± 47	41.5	<0.001
C5b-9 (AU/ml)	311 ± 68	38 ± 7.2	147 ± 39.6	51 ± 11.7	11.9	<0.001
FXIIa-AT (µmol/l)	14.0 ± 0.98	14.8 ± 2.0	5.4 ± 1.7	5.8 ± 2.0	15.6	0.001
FXIa-AT (µmol/l)	3.63 ± 0.39	3.91 ± 0.36	0.34 ± 0.12	0.36 ± 0.11	72.4	0.001
TAT (µg/ml)	21,839 ± 2,850	27,286 ± 3,545	4,467 ± 2,285	2,298 ± 854	29.4	<0.001
β-TG (IU/ml)	6,778 ± 299	6,991 ± 738	4,904 ± 694	5,166 ± 264	5.9	0.01
MPO (ng/ml)	23.2 ± 6.44	17.2 ± 3.7	17.1 ± 3.7	10.2 ± 2.2	2.8	0.122
Insulin (pmol/l $\times 10^3$)	33.6 ± 7.3	40.9 ± 9.4	44.4 ± 14.0	35.1 ± 6.4	1.0	0.692

Data are *n*, means ± SE, *F*, or *P*. AU, arbitrary units.

The rapid decrease in platelet counts and the subsequent fall in PMNs and monocytes, together with the histological finding of PMNs and monocytes accumulating around an inner layer of P-selectin–positive platelets, provide strong evidence that the process is initiated by platelets. Presumably, the PMNs and monocytes bind to the islets via the sialyl-Le^x to P-selectin on platelets adhering to the islet surface (21,22). Another explanation for the dramatic decrease in CD11b$^+$ leukocytes could be that CD11b/CD18 mediates binding to fibrin, resulting in CD11b$^+$ cells being captured in the developing fibrin lattice.

The generation of FXIIa-AT, FXIa-AT, and TAT during islet perifusion suggests that activation of the coagulation cascade occurred through the intrinsic pathway. Platelets have previously been reported to be capable of directly activating both factor XII and XI in response to collagens (23,24). Coagulation via the extrinsic pathway cannot be completely excluded, even though the upregulation of tissue factor on activated monocytes is far too slow a process to explain this very rapid activation of coagulation (25). Even so, extrinsic activation may be important later in the reaction sequence: monocyte binding to P-selectin on platelets has been shown to upregulate tissue factor after ~1 h (26).

A marked degree of complement activation also occurred, as indicated by the extensive increase in C3a and the formation of sC5b-9. This activation was presumably not triggered by the islets themselves but occurred secondarily to the previous events. This assumption is supported by the fact that complement activation began after platelet consumption and coagulation had already occurred and that immunoglobulins and complement components (C3c and C1q) were not present on the islet surface. Previous reports that islets are not damaged by fresh human serum during in vitro culture also support this notion (27). Although complement activation is unlikely to directly damage the islet cells through C5b-9–mediated cytolysis, the generation of proinflammatory products (C3a and C5a) might potentiate the inflammatory reaction toward the islets. C3a and C5a are potent proinflammatory mediators that can activate granulocytes and monocytes to release cytokines, upregulate complement receptors, and cause an influx of these cells to the site of islet implantation (28,29). In addition, complement activation products, particularly sC5b-9, can further enhance platelet activation (30,31).

Addition of sCR-1 resulted in a nearly complete inhibition of complement activation, with C3a and sC5b-9 levels similar to background levels; however, the consumption of platelets, neutrophils, and monocytes remained unaffected. Macroscopic coagulation and the increase in FXIIa-AT, FXIa-AT, and TAT were also unchanged. Thus, it appears that complement activation was not directly responsible for activating platelets or for initiating coagulation and cellular activation. Furthermore, considering that the clustering of PMNs and monocytes around the islets was not influenced by sCR-1 treatment, it is unlikely that complement fragments were the ligand for these cells.

In parallel with the binding of platelets to the islets, we observed a rapid liberation of insulin during the first 5 min. Thereafter, insulin release occurred at a more moderate rate. Complement-mediated damage may have accounted for this rapid release of insulin; however, both the time course and the lack of complement activation fragments on the islet surface argue against this possibility. Release of insulin as a result of glucose stimulation is also highly unlikely, because the glucose concentration in the blood never exceeded 7.4 mmol/l. A thin layer of platelets and fibrin could still be observed on the islet surface when sCR-1 plus heparin was added to the blood, and the rapid release of insulin from the islets did not decrease, suggesting that it is caused by factors released from the adhering platelets. The most likely platelet mediators are Ca^{2+}, ATP, and ADP, all of which are known to stimulate insulin release (32,33).

The disruption of normal islet morphology that we observed after ~15–30 min, with condensed islet cell nuclei and infiltration of a large number of CD11b$^+$ cells, occurred in parallel with the consumption of PMNs and monocytes. In addition to platelets, both neutrophils and monocytes are potential effector cells for islet damage, releasing a wide range of tissue-damaging enzymes and radicals known to be toxic to β-cells (9,34,35). Moreover, P-selectin exposed on activated platelets can also stimulate monocytes to secrete

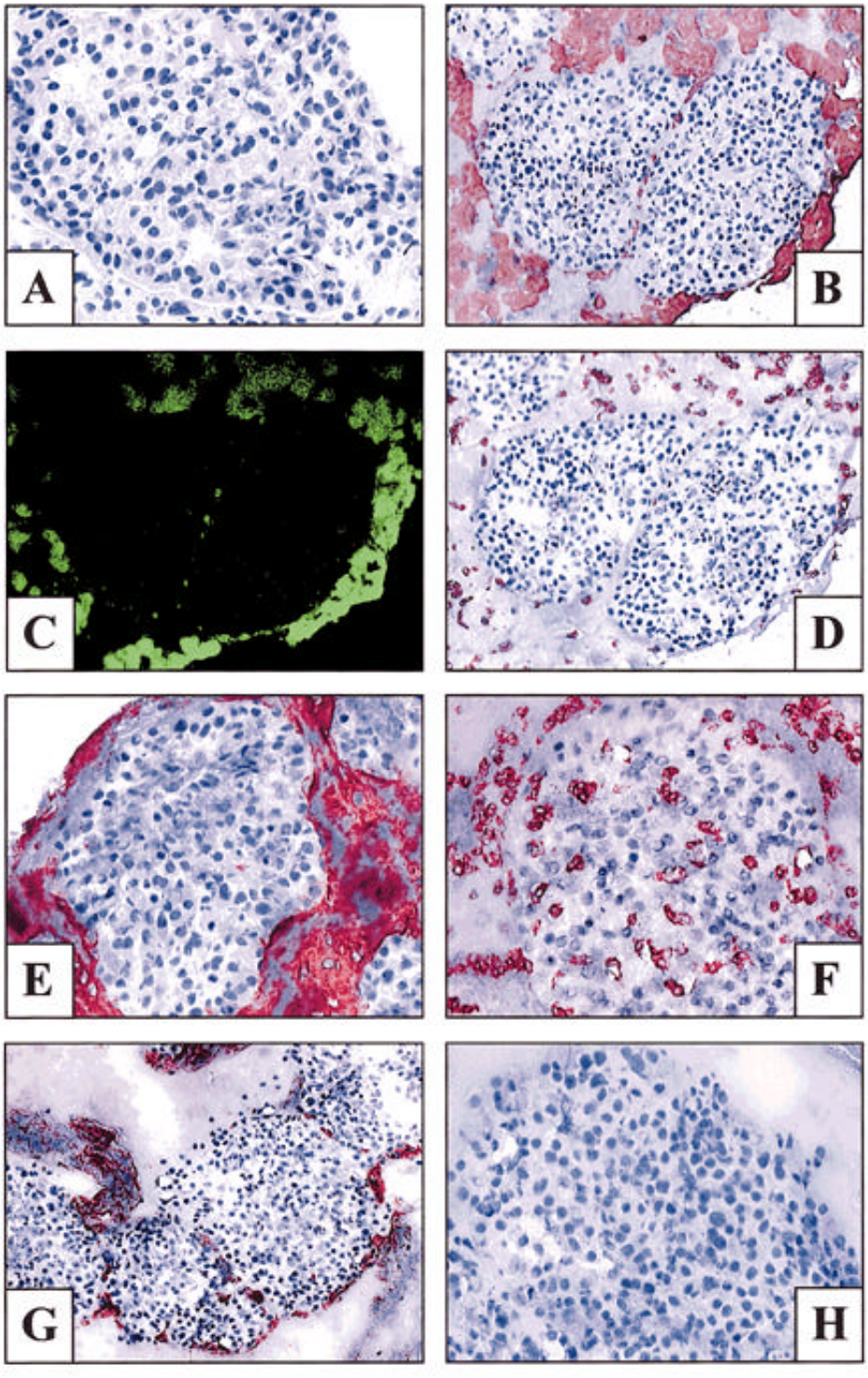

FIG. 2. Well-preserved morphology of human islets before perfusion (*A*). Consecutive sections of human islets retrieved after 5 min in blood and stained for the presence of CD41 (*B*), P-selectin (*C*), or CD11b (*D*) revealed rapid adhesion of activated platelets on the surface of the islets. PMNs and monocytes were found to accumulate in the thrombi surrounding the islets (*D*). At 60 min, a firm capsule of fibrin and platelets (*E*; CD41) surrounded the islets, which were infiltrated with PMNs and monocytes (*F*). These findings were counteracted when the blood was supplemented with sCR-1 in combination with heparin: only a thin capsule of platelets partially covered the surface of the islets (*G*), with no infiltration of CD11b$^+$ cells (*H*).

chemokines that are deleterious to islets, and islet β-cells are also highly sensitive to free radicals because of the low levels of radical-scavenging enzymes in the islets (36,37). The physical entrapment of the islets by platelets, neutrophils, and monocytes in a macroscopic blood clot may enhance the local action of factors toxic to β-cells. Furthermore, islet entrapment in blood clots has been shown to delay revascularization and engraftment (38).

After intraportal pig-to-pig islet transplantation, the morphological findings were indeed similar to those seen with human islets perfused in vitro. Moreover, similar findings have been observed in a patient transplanted intraportally with human islets. A liver biopsy obtained from one patient 2 days after transplantation revealed an intraportal thrombosis composed of fibrin strands and leukocytes clustering around islet cells (39). The authors referred to this finding as an islet cell "thrombus." In addition, intense portal inflammation was seen 2 and 5 days after transplantation.

The inflammatory reaction that occurred when islets were exposed to blood was deleterious to at least some of the islets, and as a result of this initial strong inflammatory response, subsequent antigen presentation would probably be

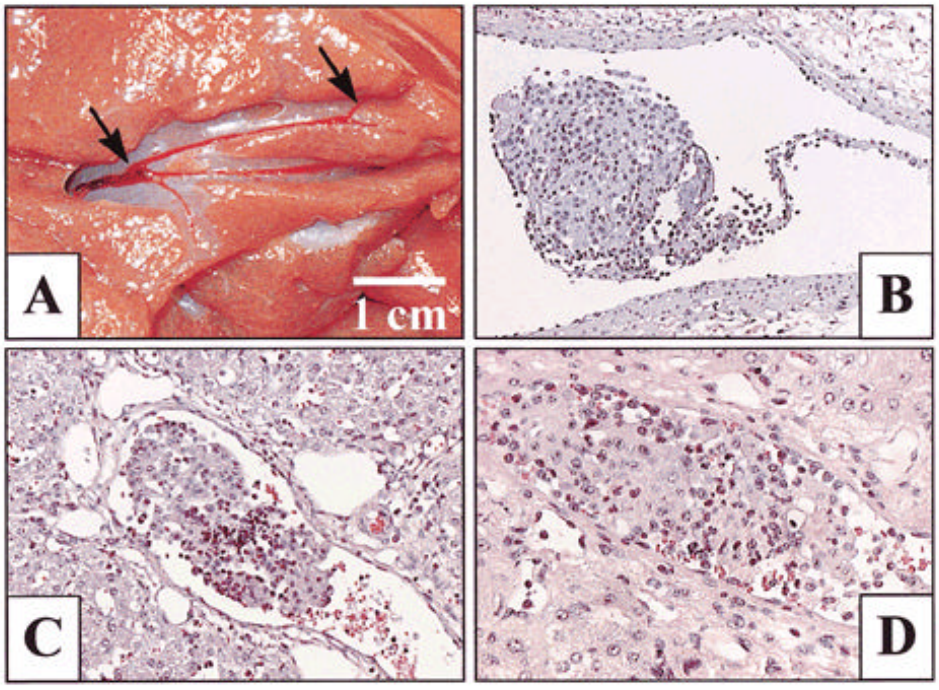

FIG. 3. Exposure of the portal tree at 60 min after intraportal adult porcine islet allotransplantation revealed thrombi at multiple sites. Most thrombi branched off deep into the portal tree (*A*). Hematoxylin- and eosin-staining of liver biopsies revealed thrombus formation in many portal veins, some of which contained entrapped porcine islets (*B–D*). Some islets seemed to be surrounded by platelets and fibrin, occasionally with a long fibrin tail extending away from the islet (*B*). The entrapped islets all showed a disrupted morphology (*C, D*).

promoted, leading to an accelerated and reinforced cell-mediated immunity in a later phase (cf. the role of adjuvants in immunization) (29,40–44). This may explain why the results after islet transplantation in patients with type 1 diabetes, which occurs across both an autoimmune and an allogenic barrier, are dramatically inferior to the results seen in patients who have undergone autologous islet transplantation after pancreatectomy, a situation in which no immune barrier exists (3).

Most centers performing allogenic islet transplantation today use systemic heparin at the time of transplantation. Heparin is usually administered as a bolus dose of ~75 IE/kg BW, corresponding to ~5,000 IE for a 70-kg person (i.e., 1 IE/ml blood). With a half-life of 1–2 h, heparin is active for only a few hours. Addition of heparin (4 IE/ml blood) in the present study prevented coagulation, reduced cell consumption, and, to a large degree, inhibited complement activation; however, islet morphology still revealed extensive platelet and fibrin formation around the islets as well as a marked infiltration of $CD11b^+$ cells. Only the addition of heparin in combination with sCR-1 effectively inhibited coagulation and complement activation, and more importantly, preserved islet morphology.

In clinical islet transplantation, the total number of islets given to a 70-kg patient is ~600,000 IEQ (8,000–9,000 IEQ/kg BW). This number corresponds to a total islet surface area of ~400 cm^2. This large islet surface area is slowly introduced into the portal vein, where the low pressure and slow flow provide optimal conditions for interaction with hematological cells as well as the cascade systems in the blood.

Based on the results of the present study, we postulate that a substantial fraction of the islets transplanted via the portal vein into the liver will be damaged shortly after transplantation. Moreover, the strong inflammatory reaction, induced by activation of the coagulation and complement systems, will enhance a subsequent acquired immune response. Strategies to efficiently inhibit these cascade reactions at the time of transplantation and during the first postoperative days may be of great importance in improving the outcome of clinical islet transplantation.

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