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Isolated Human Islets Trigger an Instant Blood Mediated Inflammatory Reaction: Implications for Intraportal Islet Transplantation as a Treatment for Patients with Type 1 Diabetes

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ABSTRACT

Islet transplantation offers a logical means to treat insulin-dependent diabetes. However, for reasons poorly understood, the clinical results with islet transplantation have been vastly inferior to those obtained with whole organ pancreas transplantation. The conventional technique for transplanting isolated islets is by intraportal injection, with the islets being trapped in the liver.

Human islets exposed to human blood trigged an "instant blood mediated inflammatory reaction", IBMIR, characterised by platelet consumption, and activation of the coagulation and complement systems. The islets became surrounded by clots and infiltrated with leukocytes, and there was evidence of islet damage as reflected in insulin dumping. When heparin and a complement inhibitor (SCR1), was added to the system, IBMIR was suppressed and islet damage reduced. After intraportal pig-to-pig islet intraportal allotransplantation similar morphological changes was found, corroborating the in vitro findings.

Thus, IBMIR inflicts a significant damage to human islets exposed to human blood and IBMIR will also, most likely, enhance the subsequent specific, cell mediated, rejection. Platelet and complement activation seem to be the most important factors in the pathogenesis of IBMIR. The results presented strongly suggest that IBMIR observed both in vitro and in vivo when isolated islets come in contact with blood could provide an explanation for the unsatisfactory results seen in clinical islet allotransplantation.

INTRODUCTION

Combined kidney-pancreas transplantation has become a valid therapeutic option for the treatment of type 1 diabetic patients with end-stage diabetic nephropathy [17]. Pancreas transplantation restores metabolic control and improves quality of

life. Even more importantly, it reduces the high mortality rate usually found in this group of patients [19]. Ten years after kidney-pancreas transplantation 80% of the patients are still alive, in sharp contrast to the 20% survival rate of those receiving a kidney alone. The mortality rate in the patients with combined pancreas and kidney transplantation was reduced, mainly because of the lower risk for cardiovascular disease. These findings led to the conclusion that combined pancreas and kidney transplantation should be considered for all patients with type 1 diabetes who have end-stage diabetic nephropathy who qualify for kidney transplantation. Pancreas transplantation can, however, only be offered to a sub-population of diabetic patients, mainly due to the surgical risk and the post-operative complications.

Previous experience with islet transplantation has demonstrated that although the procedure is safe, the clinical outcome is markedly inferior when compared with pancreas transplantation. To date, 405 adult human islet transplantations have been performed worldwide. Of the 200 recipients treated since 1990, only 8% were insulin-independent 1 year after transplantation. In contrast, insulin independence was achieved at 1 year in 22 % of the patients in a subgroup of 60 in whom 1) the preservation time for the islets was <8 h, 2) more than 6,000 islets/kg were transplanted, 3) the islets were transplanted to the portal vein, and 4) induction immunosuppression involved administration of antilymphocytic antibodies. Fewer than 10 centers worldwide are actively pursuing islet transplantation at this time. Currently there are 7 patients who are insulin-independent at 1 to 4 years after transplantation. (data from the International Islet Transplant Registry, Newsletter 8, 1999).

The reasons for the poor results that have been obtained with clinical islet transplantation have remained an enigma for the past 20 years. The histocompatibility barrier, the underlying autoimmune disease, and the immunosuppressive agents used are the same as in pancreas transplantation. However, one major distinction is that while the islets remain protected from the recipients blood by the intact endothelial cell lining of the native blood vessels during pancreas transplantation, transplantation of islets usually entails the injection of the isolated islets into the portal vein, where the islets come into direct contact with whole blood. In clinical islet transplantation, the total number of islets given to a 70-kg patient is about 600 000 IEQ (8,000–9,000 IEQ/kg BW). This number of islets has an approximate surface area of 400 cm^2. The islets are injected into the portal vein, where the low pressure and slow blood flow provide optimal conditions for interaction with the cells and cascade systems in the blood. Although several other transplantation sites have been evaluated in experimental models, intraportal transplantation is the only site established thus far for clinical islet transplantation.

Thus far, little attention has been paid to the possibility that islets transplanted into the blood stream may elicit an injurious incompatibility reaction. We have studied this possibility both in a human allogeneic in vitro system and in vivo in a pig islet allotransplantation model. An islet perfusion system was developed that

mimics the situation in vivo immediately after transplantation of islet cells into the portal vein and have used this system to examine the effect of whole blood upon transplanted islets. Isolated human islets were exposed to fresh, non-anticoagulated ABO-compatible human blood in polyvinyl chloride (PVC) tubing loops whose inner surface was coated with covalently bound heparin. When human islets were exposed to human blood in the loop, a series of destructive thrombotic/inflammatory events took place. This inflammatory reaction has not been described in detail previously and therefore lacks a descriptive name. We have chosen to name it an Instant Blood Mediated Inflammatory Reaction or "IBMIR".

THE ROLE OF PLATELETS IN IBMIR

The most striking event observed was the appearance of macroscopic clotting within 5 min after introduction of human islets into the blood. There was a rapid loss of platelets from the blood concomitant with binding of platelets to the islet surface, followed by a continuous fibrin formation that generated a capsule surrounding the islets. Simultaneous up-regulation of P-selectin and secretion of ß-thromboglobulin indicated platelets activation. These events point to a platelet-driven reaction directed against the islets. The ligand/s to which the platelets bind are still not identified. The rapid decrease in platelet counts was followed by a drop in PMN and monocyte counts, together with a morphological observation of PMNs and monocytes clustering around the islets. After approximately 15 to 30 min, in parallel with the consumption of PMNs and monocytes, a disruption of normal islet morphology was observed with the appearance of condensed islet cell nuclei and infiltration of a large number of $CD11b^+$ cells.

Under physiological conditions platelet activation is under rigorous control by endothelial cells that inhibit platelet aggregation by several mechanisms. All these regulatory systems are most likely absent on the islet surface. Hence, the islets are unable to regulate platelet activation.

The process of IBMIR is very similar to the platelet activation that occurs in blood in response to vascular injury, i.e. upon contact with connective tissue in the subendothelial matrix or with a biomaterial (e. g. PVC or titanium) which binds fibrinogen to the surface [9]. When islets are infused into the bloodstream via the portal vein, a similar reaction probably occurs: collagen types I, III, IV, and V have been reported to surround human islets, and collagen is known to mediate platelet binding and activation [20, 21]. The $\alpha_2ß_1$ glycoprotein Ia–IIa complex, CD36, P65, and GPVI have all been proposed as platelet collagen receptors [10]. Another possibility is that membrane structures or plasma proteins which are ligands for platelet receptors (e.g. fibrionogen or von Willebrand factor) bind to the islet surface when it is exposed to blood. Our previous studies have not defined which of these mechanisms are involved in triggering the platelet/islet event. Preliminary electron microscopy data have shown that the surfaces of islets cultured for 3–4 days show only scarce amounts of connective tissue elements.

THE ROLE OF COAGULATION IN IBMIR

The coagulation system seems to be activated by the intrinsic pathway when islets are mixed with blood in the in vitro model. It is feasible that the negatively charged islet surface activates this coagulation pathway. If so the intrinsic pathway of activation could be a potential activator of the whole reaction, since small amounts of fibrin would be able to bind and activate platelets. Platelets have been suggested to activate the intrinsic pathway and factor XI has been reported to be activated in response to collagen [22, 23]. Coagulation via the extrinsic pathway cannot be completely excluded from consideration, even though tissue factor was not expressed by the islets, and the up-regulation of tissue factor on activated monocytes is far too slow a process to explain this very rapid activation of the coagulation system [1]. However, the extrinsic activation may be important later in the reaction sequence. For example, monocyte binding to P-selectin on platelets has been shown to upregulate tissue factor after approximately 1 h [5]. There are also reports that immune complexes and complement (e. g. C5a) are able to enhance expression of tissue factor on monocytes [12].

THE ROLE OF COMPLEMENT IN IBMIR

Complement activation via the classical pathway (triggered by immune complexes) and the alternative pathway (triggered by "non-self") leads to formation of a complex composed of C5b-9. C5b-9 forms a "channel" through the plasma membrane, leading to cell lysis and eventually cell death. Another main function of the complement system is to generate the anaphylatoxins C3a and C5a, which are extremely potent proinflammatory mediators that can also potentiate the thrombotic reaction. A marked degree of complement activation also occurred when isolated islets were exposed to human blood, as indicated by an extensive generation of C3a and sC5b-9. This activation was presumably not triggered by the islets themselves but occurred secondarily to the previous events. This assumption was supported by the fact that complement activation began after platelet consumption was almost completed and coagulation activation had already started. A number of observations, however, suggest that the complement system does not directly contribute to the destruction of the islets. Other investigators have failed to detect complement activation by islets after exposure to human serum [8], despite the fact that islet cells do not express the complement regulatory proteins DAF (CD55) and MCP (CD46) and have only low amounts of protectin (CD59) (unpublished observations). These findings are in agreement with what we have seen when islets are exposed to whole blood since in the initial phase we have no evidence of complement activation on the islet surface. Although complement activation is unlikely to cause direct damage to the islet cells through C5b-9 cytolysis, the generation of proinflammatory products (sC5b-9, C3a, and C5a) most likely potentiates the inflammatory reaction toward the islets. C3a and C5a are powerful chemoattractants for PMNs and monocytes and can stimulate an influx of these cells to/into the islets [2]. Activation

through the C5a receptor causes enzyme release (e. g. myeloperoxidase and elastase) by granulocytes and cytokine release (IL-1, IL-6, IL-8 and TNF-α) by monocytes. Another important effect of C5a is that it up-regulates complement receptor 3 (CD11b/CD18) on PMNs and monocytes. C5a stimulates endothelial cells to release heparin sulfate, upregulate tissue factor, secrete von Willebrand factor and express P-selectin. These changes favour fibrin deposition, augmented thrombin-mediated platelet aggregation and adhesion of PMNs. Soluble C5b-9 (sC5b-9) triggers endothelial cells to express tissue factor and a number of adhesion molecules [18]. In addition, complement activation products, particularly sC5b-9, can further enhance platelet activation [15]. Thus complement activation might induce direct inflammatory effects but also indirect effects mediated by the endothelial cells in the portal system.

INSULIN "DUMPING" DURING IBMIR

During the first 5 min after addition of the islets to the blood in the loops and in parallel with the binding of platelets to the islets we observed a rapid liberation of insulin, followed by a release at a more moderate rate. Release of insulin as a result of glucose stimulation was unlikely in this case, since the glucose concentration in the blood never exceeded 7.4 mmol/L. Complement-mediated damage was a potential cause of this rapid release, but both the time course and the lack of complement activation products on the islet surface argued against this possibility. The most likely cause of insulin release are, however, mediators released from activated platelets such as Ca^{2+}, ATP, and ADP, all known to stimulate insulin release. In addition to platelets, PMNs and monocytes are potential effector cells in islet damage. P-selectin exposed on activated platelets, released PAF, and the fibrin lattice can all stimulate monocytes and PMNs to release a wide range of tissue-damaging enzymes and radicals known to be toxic to ß-cells [6, 13, 16]. Islet ß-cells are highly sensitive to secreted chemokines and to free radicals because of the low levels of radical-scavenging enzymes in the islets [11, 24]. Furthermore, 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 ß-cells.

CLINICAL AND EXPERIMENTAL ALLOGENEIC INTRAPORTAL ISLET TRANSPLANTATION

Previous case reports have described a phenomenon similar to what we have observed in the in vitro loop model. Several years ago, the Ricordi group published a report of patients transplanted intraportally with human islets [14]. Liver biopsies obtained from one of the patients two days after transplantation revealed an intraportal thrombosis composed of fibrin strands and leukocytes clustering around the islet cells [14]. The authors referred to this finding as an "islet cell thrombus". In addition, intense portal inflammation was seen two and five days after transplantation. Similar findings were observed in the other four patients. They interpreted

their findings as part of a process of eliminating contaminating non-endocrine tissue. Based on our findings in the loop model we challenge this view and suggest that the observed destructive process is unable to discriminate between endocrine and non-endocrine tissue.

After intraportal pig-to-pig islet allotransplantation, we have also found morphological changes similar to those reported by Sever et al. [14]. Liver biopsies from these pigs show islets entrapped in fibrin strands, which embolize in the branches of the portal tree [3]. These in vivo observations clearly confirm our findings from the loop system.

THE EFFECT OF HEPARIN AND SOLUBLE RECOMBINANT CR1 (SCR1) IN IBMIR

Addition of 4 IU/mL of heparin to the blood in the loops inhibited the macroscopic clotting and the generation of FXIIa-AT, FXIa-AT, and TAT. As a result of the decreased fibrin generation, the consumption of platelets, PMNs, and monocytes was abrogated.

A reciprocal effect was obtained by the addition of the recombinant form of the complement inhibitor soluble complement receptor 1 (sCR1). Addition of sCR1 resulted in a nearly complete inhibition of complement activation, with C3a and sC5b-9 levels similar to the background levels. However, the consumption of platelets, neutrophils and monocytes remained unaffected, as were the macroscopic coagulation and the increase in FXIIa-AT, FXIa-AT, and TAT. Thus, it appears that complement activation was not directly responsible for the platelet activation or for initiating coagulation and cellular activation. Furthermore, since the clustering of PMNs and monocytes around the islets was not influenced by sCR1, the complement fragments were apparently not the ligands to which the cells were binding.

In contrast, the treatment with a combination of both inhibitors (i. e. heparin and sCR1), prevented disruption of islet morphology and leukocyte infiltration of the islets a finding which strongly suggests that the reaction is driven by both cascade systems. Despite this preventive effect of the two drugs, a thin layer of platelets and fibrin could still be observed on the islet surface, and the rapid release of insulin from the islets was not diminished. This observation further supported the hypothesis that the initial event in the reaction was triggered by the binding of platelets to the islet surface and that the insulin release indeed was caused by factors from the adhering platelets.

STATE-OF-THE-ART TRANSPLANTATION IN RELATION TO IBMIR

At present most centers performing allogeneic islet transplantation use low-dose systemic heparin at the time of transplantation. Heparin is usually administered as a bolus dose of approximately 75 IE/kg body weight, corresponding to approximately 5000 IE for a 70-kg person or 1 IE/ml of blood. The initial blood concentration is

25% of the concentration we used in the loop model, and given its redistribution in the body fluid and its half-life of 1 to 2 h, the heparin concentration in patients is maintained for only a very short time. The heparin concentration in the clinical setting is far from the concentration of 4 IE/ml heparin that we used in the in vitro model, a concentration that prevented coagulation and reduced cell consumption. Furthermore, since the addition of heparin alone was unable to prevent extensive platelet and fibrin formation around the islets as well as a marked infiltration of $CD11b^+$ cells in our in vitro system, we conclude that only the use of a high heparin concentration in combination with sCR1 can effectively preserve islet morphology. Such a high concentration of heparin is, however, not acceptable for long-term use in patients, particularly in those with cardiovascular complications often associated with diabetes. It is therefore important that we gain a better understanding of mechanisms involved in this undesirable inflammatory reaction and develop clinically acceptable methods to prevent to this process.

IMMUNOSUPPRESSION IN RELATION TO IBMIR

Immunosuppressive drugs given to prevent T cell-mediated allograft rejection are most likely not capable of preventing IBMIR. In addition to the acute thrombotic/inflammatory destructive reactions which are deleterious to the islets, antigen presentation may also be promoted, leading to an accelerated and reinforced cell-mediated immunity at a later stage in the process [4, 7]., c.f. the role of adjuvants in immunization. This could explain why the results after intraportal islet transplantation to 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, a situation in which no immune barrier exists (data from the International Islet Transplant Registry, Newsletter 8, 1999). If IBMIR were counteracted, a larger islet mass would engraft and escape subsequent specific immune responses, thereby markedly improving the outcome in clinical islet transplantation.

CONCLUDING REMARKS

The detrimental effects of IBMIR on the clinical outcome of islet transplantation in patients with type 1 diabetes could provide an explanation for the relatively low success rate of this procedure. However, the use of a high number of islets (>10 000 IEQ/kg recipient) and an extensive immunosuppressive protocol may in some patients overcome the effects induced by IBMIR. Nevertheless, our results strongly suggest that the inflammatory events observed both in vitro and in vivo when isolated islets come in contact with blood could provide an explanation for the unsatisfactory results seen in islet allotransplantation.

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