ABO expression on islet of Langerhans cells and activation of the complement system

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Abstract

Islet of Langerhans cells are readily destroyed once transplanted to a Type 1 diabetic despite being ABO compatible. The complement system and the coagulation cascade play a role in this destruction.

My project involves investigation of blood group antigens expressed on both paraffin embedded islets using immunohistochemistry techniques and on fresh human and pig islets using the Complex Object Parametric Analyser and Sorter (COPAS) and the confocal microscope. Optimisation of various immunohistochemistry methods allowed ABO, endothelial cells and collagen staining patterns to be visualised. Fresh islets were analysed in the same manner using FITC conjugated antibodies and COPAS analysis. Islets were also incubated with autologous, compatible and incompatible plasma to assess if there was a difference in blood group, IgG, IgM and C3c binding.

Conclusions drawn seem to suggest that the islets used for transplantation are contaminated with exocrine parts rich in ABO and collagen antigens and that binding of complement factors rise when islets are incubated in compatible plasma as well as incompatible plasma.
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1.0 Introduction

1.1 Diabetes Mellitus

The two most common forms of diabetes are characterised by a decrease or complete absence of the production of insulin (type 1 diabetes), or decreased sensitivity of body tissues to insulin (type 2 diabetes). Insulin allows glucose enter cells so that it can be utilised as a source of energy. In the beta cell proinsulin is cleaved into active insulin and C-peptide. C-peptide is stored in secretory granules within the β cells and released into the blood stream in amounts equal to that of insulin. Figure 1A depicts how a normal functioning pancreas should respond to varying glucose levels. If blood glucose levels rise, the beta cells of the pancreas release insulin which stimulates the fat cells to utilise this excess energy\(^1\). This mechanism is lost in type 1 diabetes. It is an autoimmune disorder in which autoimmune reactive T cells attack the beta cells in the islets of Langerhans cells of the pancreas destroying them. The autoimmune attack may be triggered by a viral infection. There is also evidence that genetic vulnerability plays an important role in the inherited tendency to develop type 1. Type 1 is treated with insulin replacement therapy, usually by injection or insulin pump, carbohydrate counting and careful monitoring of blood glucose levels using glucose metres\(^2\). There are several different types of insulin on the market today classified according to their speed and duration, rapid acting, short acting, intermediate acting and long acting. The various types of insulin can be used in combination to achieve around the clock glucose control.

Figure 1A showing which cells are stimulated when glucose levels fluctuate\(^5\)
Islet cell regeneration, use of stem cells, generation of an artificial pancreas, genetically engineering an insulin gene and shutting down the auto reactive T cells that attack beta islet cells are other potential cures for diabetes type 1. Untreated diabetes can lead to coma, ketoacidosis and in extreme cases death. Retinopathy, renal damage, vascular damage, neuropathy and hypoglycaemia are some of the risk factors associated with type 1 diabetes\textsuperscript{3}.

1.2 Pancreas Transplantation as a potential treatment for diabetes type 1

The pancreas is essential both for digestion and regulation of metabolism. The exocrine part secretes digestive enzymes and bicarbonate into the intestine while the islets of Langerhans cells in the endocrine part produce several hormones which regulate carbohydrate metabolism. A whole pancreas transplant carries with it an associated risk of leakage of digestive enzymes into the stomach. For patients with kidney failure a pancreas transplant is a viable option as both pancreas and kidney transplant can be carried out simultaneously. There is an 85% success rate if both the pancreas and kidney are transplanted at the same time, however very sick patients cannot undergo such a major operation\textsuperscript{4}.

The first approach to replacing beta cells as a cure for diabetes type 1 took place in 1966 when a whole pancreas was transplanted to a diabetic recipient in the University of Minnesota. The survival however was less than 3 months\textsuperscript{4}. Early technical issues that needed to be resolved included the management of exocrine drainage of the pancreas allograft, location of the venous drainage of the allograft, the prevention of graft thrombosis and timing of the pancreas transplant relative to the renal transplant.
For isolated pancreas transplants the graft is ideally placed on the right side, where vascular arrangement of the recipient is more favourable. The pancreas is typically bladder drained and a final opening to the peritoneum is created to help prevent the accumulation of peripancreatic fluid collections in an extra-peritoneal space. Post-operatively when the patient is tolerating a liquid diet, oral immunosuppressive agents are introduced. Prophylactic antibacterial antibiotics are continued for 5 days post-operatively. Evidence of rejection may be as subtle as fever or pain over the allograft. Hyperglycemia is usually a late sign of rejection and may also indicate thrombosis⁴.

1.3 Background history of islet transplantation through the ages.

From the late 19th century until the present day, the transplantation of islet of Langerhans cells has been considered as a potential therapy for type 1 diabetes. Paul Langerhans identified pancreatic islets in 1869 describing them as discrete islands or islets surrounded by pancreatic exocrine tissue. The first approach to using islets as a cure for diabetes took place in 1894 when Williams used minced sheep’s pancreas and extracts of pancreas in glycerine for oral and subcutaneous therapy⁶. In the 1980’s reports of successful allogeneic islet transplantation in patients with type 1 diabetes, using immunosuppressive therapy and purified human islets came about, Paul Lacy pioneering the movement⁷. Internationally however the overall success rate was reported as less than 10 percent. In 1992, Pyzdrowski et al reported that 265,000 islets were sufficient to establish insulin independence. In 1995, Wahoff et al reported an insulin-independence rate of 74 percent two years after autologous islet transplantation in 14 patients who had undergone total pancreatectomy and who had received a portal-vein infusion of more than 300,000 islets. By the year 2000, a 100 percent success rate was reported by Shapiro et al⁸.
This was a huge break through for islet transplantation and was named the Edmonton protocol. All 7 recipients showed insulin independence with no rejection episodes\(^8\). Each recipient had normal glycosylated haemoglobin values after transplantation as well as detectable C-peptide values at 3 and 6 months after transplant\(^4\). Avoidance of corticosteroids, which can be toxic to islets, and the use of the anticytokine drugs also helped rise this success rate. The recipient must undergo harsh immunosuppressive therapy before the transplant can take place. The Edmonton protocol uses a combination of immunosuppressive drugs including dacliximab, sirolimus and tacrolimus. Dacliximab is given intravenously right after the transplant and is then discontinued. The other two must be taken for life as they keep the immune system from destroying the transplanted islets. These drugs are quite toxic and can cause side effects such as oral ulcers, anemia, fatigue and hypertension. The risk of renal damage is also quite high. In 2002, the same group followed 17 islet recipients for 34 months. This study showed that 80% of the patients were insulin independent after one year and 67% after more than 2 years\(^7\). A reason for this vast improvement in islet transplantation may have been the use of many donors to attain a transplanted islet mass sufficient to achieve normal glucose levels and independence from exogenous insulin. Islet transplantation can relieve glucose instability and problems with instability. One study carried out by Ryan et al outlines that C-peptide secretion is maintained in the majority of patients for up to 5 years, although most revert to using some insulin\(^9\).

### 1.4 Islet cell transplantation as a potential treatment for diabetes type 1

Modern islet isolation technology involves the procurement of a healthy pancreas from a brain-dead donor whose heart is beating. In the period from 1999 to 2004, 471 patients
with type 1 diabetes have received islet transplants at 43 institutions worldwide. Islets have a five to ten times higher blood perfusion than the surrounding exocrine tissue. This enables cells to have a high basal metabolism and allows for a rapid delivery of hormones to the blood stream. Adult pancreatic islets have a dense capillary network and approximately 10% of the islets consist of blood vessels. Endothelial cells line all blood and lymphatic vessels. For an average size person (70kg), a typical transplant requires about 1 million islets, isolated from two donor pancreas. Islet cells constitute only 1% of the whole organ. During isolation the pancreas duct is cannulated and collagenase is infused to separate islets from exocrine and ductal tissue. The whole organ is then placed in an oscillating metal chamber that contains beads. When the oscillating step is complete, the exocrine and endocrine cells are purified by density gradient centrifugation. The final product is evaluated for purity and viability before it is transported to the angiography suite for transplantation. The isolated islets are transplanted to an ABO compatible recipient through the portal vein in the liver. An overview of this process is depicted in figure 1B. Revascularisation is critical for the long-term survival of transplanted cells. Endothelial cells in the islets have been shown to contribute to this process. An obvious benefit of this sort of transplantation is the avoidance of the invasive surgical procedure required for a solid organ transplant. This would allow the more debilitated patients to undergo a procedure to

![Figure 1B showing pathway of islets from donor pancreas to recipient portal vein](image)
reverse diabetes\textsuperscript{4}. The patient’s body will however treat the new cells as it would treat the introduction of any other foreign tissue. The immune system will attack the cells so immunosuppressants are needed. If the number of islets from one donor is not sufficient, often islets from several preparations are used. The health of and the medications used by the potential donor, the care with which the surgical team removes the pancreas and the efficiency of transportation to the laboratory are important variables. Once the pancreas reaches the lab, 4-6 hours are required for complete processing and quality assessment of the islets. Some argue that the purification step should be abolished as it adds time and can cause the loss of 30-50\% of islets. Unpurified islets have been used successfully in autologous transplantation. Purification removes the pancreatic-duct stem cells that may give rise to new islets after transplantation. At the moment the only candidates for islet transplantation are renal failure patients. The risks associated with placing such patients on immunosuppressants must be weighed against the benefits of transplanting islets. An islet transplantation carries with it risks of intra-abdominal hemorrhage and portal vein thrombosis. The fact that there is already a good alternative to islet transplantation (i.e. the modern intensive insulin regimen) forces us to regard any newer, riskier interventions with a critical eye. Other concerns relating to the field include questions about the impact of having insulin-producing foreign cells within the hepatic parenchyma, the long-term consequences of elevated portal pressures resulting from the islet infusion, and the fact that islet recipients can be sensitized against donor tissue types, making it more difficult to find a suitable donor should another life-saving transplant be required in the future. Also, very few islet transplant recipients have remained euglycemic without the use of any exogenous insulin beyond four years post-transplant. Thus, while most islet recipients achieve better glycemia control and suffer less serious hypoglycemia, islet transplantation continues to fall short of the definitive diabetes cure\textsuperscript{13}. 
1.5 Xenotransplantation

Like all transplantation therapies, islet transplantation is limited by the shortage of organ donors. The numbers are striking; at least 1 million Americans have type 1 diabetes mellitus and only a few thousand donor pancreas are available each year. The ultimate goal for islet transplantation is to find an unlimited source of cells and to be able to carry out the transplantation without the need for chronic immunosuppressive drug therapy\textsuperscript{14}. Porcine insulin is identical to human insulin in 51 out of 52 amino acids and was used routinely until recombinant human insulin was introduced in the 1980’s. In the 1990’s foetal porcine islets were successfully transplanted into 10 diabetic patients who had already received kidney transplants\textsuperscript{15}. Anti-Gal antibodies are present in all humans because of a complete lack of Gal\(\alpha(1.3)\)Gal expression. The presence of the natural anti-Gal antibodies, which accounts for \(\sim 1\%\) of circulating immunoglobulins in humans, is a major immunologic barrier in xenotransplantation preventing transplantation of pig organs or tissues into humans. As a result of the \(\alpha\)-gal epitope being expressed as millions of epitopes per cell on pig cells, including pig endothelial cells, transplantation of pig tissue results in immediate rejection. Anti-Gal antibodies of the recipient bind to \(\alpha\)-gal epitopes on the endothelial cells of the graft. This interaction activates complement, thus inducing cell destruction and platelet aggregation, ultimately resulting in the collapse of the vascular bed, ischemia and hyperacute rejection of the graft. Even if the activation of complement molecules is inhibited, the xenograft is rejected within weeks to months by anti-Gal IgG molecules. This destruction is facilitated by the antibody dependent cell mediated cytotoxicity (ADCC) process in which macrophages, granulocytes and natural killer cells bind to the Fc portion of the anti-Gal IgG molecules on the graft and exert their cytotoxic potential on the xenograft cells\textsuperscript{16}. Perhaps by using our knowledge of carbohydrate biology, we are able to alter the carbohydrate profile of cells in transgenic animals by over
expressing the H-type fucosyltransferase to reduce Galα(1.3)Gal expression. Xenotransplants of organs from α1.3GT knock-out pigs into immunosuppressed baboons have extended the life of a transplant from minutes to months\textsuperscript{15}. Encapsulation of porcine islets has shown some promise in animal models, although indefinite survival has not been achieved. The presence of an enterovirus in pigs called the perb virus has somewhat stalled the whole process of xenotransplantation. It is not exactly known if this virus can be transmitted to humans through pig islet transplantation\textsuperscript{16}.

\textbf{1.6 Transplantation Immunology}

Allogenic MHC molecules may be presented on donor Antigen Presenting Cells (APC) to recipient T cells (direct pathway), or the alloantigens may be picked up by host APC’s that enter the graft or reside in draining lymphoid organs and be processed and presented to T cells as peptides associated with self MHC molecules (indirect pathway). There are two main branches of immunity, termed innate and adaptive immunity. The innate immune system is fast acting and present since birth. It is capable of recognising highly conserved structures that are common to groups of related foreign molecules. Adaptive immunity involves specific recognition and selective elimination. Unlike innate immunity, adaptive immunity displays specificity, diversity and memory. If the innate defences are not sufficient to eliminate the intruder, the adaptive system is alerted. The constituents of the adaptive system are B and T lymphocytes. Graft rejection is mediated by T cells, including cytotoxic T lymphocytes that kill graft cells and helper T cells that cause Delayed Type Hypersensitivity (DTH) reactions, and by antibodies. In clinical transplantation, alloreactive CD4+ or CD8+ T cells or alloantibodies are capable of mediating allograft rejection.
Graft rejection is classified on the basis of histopathologic features or the time course of rejection after transplantation rather than immune effector mechanisms. These histopathological patterns are called hyperacute, acute and chronic. Pre-existing antibodies cause hyperacute rejection characterised by thrombosis of graft vessels. Alloreactive T cells and antibodies produced in response to the graft cause blood vessel wall damage and parenchymal cell death called acute rejection. Chronic rejection is characterised by fibrosis and vascular abnormalities which may represent a chronic DTH reaction in walls of arteries. General immunosuppression and minimising the strength of the specific allogeneic reaction can help to avoid or delay rejection of the graft. Immunosuppressive drugs, anti-T cell antibodies and metabolic toxins are used to inhibit and kill T lymphocytes\textsuperscript{17}.

1.7 ABO Blood Group Antigens

A, B and O blood group antigens are oligosaccharide moieties formed by glycosyltransferases. These enzymes sequentially add carbohydrate units to growing oligosaccharide chains on proteins and lipids. The specificity of the catalytic domain of these enzymes and the tissue distribution of expression determines the form of ABO blood group expressed. Hyper acute rejection occurs when incompatible organs are transplanted between incompatible blood groups. As a result immunoglobulins are produced in the absence of known antigenic stimulation. Natural antibodies are present in all humans with an intact immune response. For example a blood group A individual will have naturally occurring anti-B antibodies in their serum. However there is some evidence that normal human serum contains antibodies that react with self antigens but usually not in a pathological manner.
For example anti-A antibodies present in the serum of A blood group individuals. Naturally occurring anti-ABO antibodies play a huge role in transplantation and are the first obstacle to overcome in successful engraftments. As is the case with Anti-Gal antibodies, ABO antibodies can attack antigen sites on the transplant within minutes and activate the complement cascade, resulting in rejection. The intensity of the reaction depends on the level of antibody and antigen expression. It is hoped that one day transplantation across the ABO barrier can take place. Successful kidney transplants across this barrier have already taken place so perhaps in the future the same may be possible for islet transplants. It is thought that natural antibodies enhance phagocytosis and complement-mediated lysis by amplifying ongoing specific antibody response. Since the α-Gal epitope is very similar in its structure to blood group A and B epitopes, understanding anti-Gal response to α–gal epitopes is likely to provide information on the immune response to ABO incompatible antigens. Natural occurring anti-carbohydrate antibodies are produced by a subset of B cells expressing CD5, as opposed to adult CD5 negative B cells. As a consequence of the shortage of organs available for transplant, methods are been devised to transplant across the ABO barrier (allotransplantation). The level of antigen expression in the donor should be low. Preformed antibodies in the recipient could be removed by immunophoresis. Immunosuppressive therapy can help to target B and T lymphocytes. If high levels of the antibody appear within the first 2 weeks post transplant, the graft is usually destroyed by antibody mediated mechanism. If antibodies appear 2-3 weeks post transplant, long term graft survival is still possible, this is called accommodation. Accommodation is characterised by the survival and continued function of ABO-incompatible renal allografts in the presence of reappearing (following their initial removal) anti-ABO antibodies and an intact complement system. One review speculates that cell surface antigen remodelling and changes in the natural antibody
repertoire to fewer cytotoxic antibodies may allow this accommodation to take place. Other articles suggest that antiapoptotic genes are expressed resulting in an increased endothelial cell resistance to complement-mediated injury\textsuperscript{19}. Most of the natural antibodies produced from B cells are polyreactive i.e. can bind a variety of unrelated self and foreign antigens\textsuperscript{20}. In the \( V_H \) and \( V_L \) regions of polyreactive antibodies, there are nucleotide and amino acid sequences that are identical or closely related to germ line sequence. In contrast, monoreactive antibodies show many somatic mutations in these regions but as yet no fundamental difference in the amino acid composition between the two types of antibodies has been detected. This suggests that the answer may lie not in the linear sequence but in the three-dimensional structure of the antigen-binding pocket. This pocket is quite flexible so conformational change can occur to accommodate a particular antigen in the case of polyreactive antibodies\textsuperscript{20}. This polyreactivity can result in natural antibodies binding to an otherwise compatible graft, as in islet cell transplantation, resulting in activation of the complement cascade and destruction of the islets. Islet allotransplantation into patients with autoimmune type 1 diabetes represents a re-exposure to autoantigen. Insulin independence is not achieved in patients with autoantibody elevations. This data is consistent with a reactivation of autoimmunity that may be dependent on immunosuppression therapy and is associated with impaired graft function\textsuperscript{21}.
1.8 Collagen

Successful human islet isolation is dependent on effective separation of islets from exocrine tissue. Figure 1C depicts an islet within a pancreas. The exocrine part is very positive for collagen. A more detailed knowledge of the composition of the connective tissue of the pancreas on which collagenase is acting is vital. Previous studies have shown that collagen VI is present in the peri-islet capsule in human pancreas. Collagen I, II and IV are also present in this region. Collagen VI has been identified by immunohistochemistry as a predominant constituent within the islet exocrine interface in the human pancreas. Subtypes I, IV and V have also been found in human pancreas\(^2\). The fact that collagen VI is present in the interface is important as it has been previously shown that this subtype in its non-reduced form is resistant to digestion by bacterial collagenase unlike collagens I-V\(^2\). This may partly explain why large numbers of islets cannot be isolated from a significant proportion of human pancreases.

1.9 The Instant Blood-Mediated Inflammatory Response

The functional capacity of transplanted islets corresponds to only about 20% of that found in non-diabetic patients. This shouldn’t be the case as the tissue is transplanted in large excess. Such a low functional capacity indicates that only a small fraction of the transplanted islets are engrafting in the liver. During transplantation, the contact between the intraportal islets and the blood induces a thrombotic/inflammatory reaction called the
instant blood-mediated inflammatory reaction (IBMIR). When human islets are transplanted, there is a rapid destruction and only some islets remain. A slow destruction goes on for years, gradually destroying all islets. It is not clear what exactly causes this destruction. The IBMIR is characterised by a rapid activation of the coagulation and complement systems once human islets are infused into recipient, recruitment and infiltration of the islets by leukocytes and rapid binding and activation of platelets. This intraportal thrombosis results in clots forming in the large branches of the liver vessels, entrapping the islets and preventing them from reaching the small vessels where they can engraft. There has been a lot of work carried out regarding IBMIR and the coagulation pathway in islet transplantation, Uppsala University, Sweden leading this research. This is the only group that are investigating islets so intensely, receiving pancreas from all of Sweden, Finland, Norway and Denmark. This project will focus mostly on the role of the complement system in islet transplantation, investigating ABO, collagen and endothelial expression.

1.10 The Complement System

Complement is the name given to a system consisting of more than 35 soluble and cell-bound proteins, 12 of which are directly involved in the complement pathways. The proteins account for 5% of the serum globulin fraction. Most of these proteins circulate as inactive zymogens which undergo proteolytic cleavage. The complement proteins are synthesized mainly by hepatocytes; however, significant amounts are also produced by monocytes, macrophages, and epithelial cells in the gastrointestinal and genitourinary tracts. The central nervous system is also able to produce complement components. The products have potent biological effects and promote inflammation through chemotaxis and
increased vascular permeability. They cause cell destruction either directly through 
activation of the whole complement cascade with the formation of the membrane attack 
complex (MAC) or indirectly through a product C3b, which mediates attachment of coated 
cells to phagocytes. Once activated C3 has been produced by the classical pathway, its 
production is amplified by the alternative pathway. It is known that IgG monomer binds 
to the islets of Langerhans that produce insulin. This activates the complement system via 
the classical pathway leading to islet necrosis. The epitopes on the islet surface which the 
antibodies bind to is still unknown. IgM pentamer has been shown to bind weakly to the 
islet surface but the binding isn’t enough to activate complement.

Figure 1D: This shows the 3 pathways involved in the complement cascade. The classical 
pathway is activated by antibody-antigen complexxes, the lectin pathway by the interaction 
of microbial carbohydrates with mannose-binding proteins in the plasma or tissue fluids 
and the alternative pathway by C3b binding to microbial surfaces. Although all three 
pathways differ in their activation they all lead to the production of C3 convertase 
enzyme.
Complement triggers inflammation, opsonisation, lysis of gram-neg bacteria and cells expressing foreign epitopes attracting phagocytes to sites of infection. It also removes harmful immune complexes from the body and plays a role in the activation of naïve B-cells. The MAC can penetrate the cell membrane resulting in lysis. Membrane cofactor protein, decay acceleration factor and CD59 expressed on human cells protect them from destruction by the complement system. Transplanted islets can be destroyed by this MAC even when the recipient is ABO compatible and the islets are relatively pure. It is important that almost every step of complement activation is tightly regulated by critical fluid-phase components in association with a series of membrane-regulatory proteins and a matrix protein called decorin. The system has the potential to undergo rapid, massive activation so it is imperative that this activation is permanently under strict control. These regulatory proteins can irreversibly inactivate a specific component, maintain a protein transiently in an inactive form, or dissociate components from a multiprotein complex such as the C3 and C5 convertases. For example, C1 esterase inhibitor inactivates C1r and C1s and thereby restricts their effects to the specific site of C1 binding and activation. DAF, a membrane regulatory protein, acts by binding C3b or C4b on the cell membrane and markedly increasing the spontaneous decay of both the classical pathway C4b2a and alternative pathway C3bBb complexes. The matrix protein decorin binds to and blocks the activity of C1q. Bennet et al have described that isolated islets don’t possess these complement regulatory proteins. This would also contribute to the complement activation that is seen.
Aims

- Investigate the possibility of transplanting islet of Langerhans cells across the ABO barrier.
- Investigate ABO expression on both paraffin embedded pancreas and islet sections as well as fresh islets and analyse this using the confocal microscope and the Complex Object Parametric Analyser and Sorter.
- By using various immunohistochemistry stains, determine if exocrine parts containing collagen are remaining on islets are isolation.
- Investigate the difference in complement activation when islets are incubated with autologous plasma, compatible plasma and incompatible plasma.
2.0 Materials and Methods

**ABO Immunohistochemistry work on paraffin wax embedded islets**

2.1 Preparing paraffin wax embedded sections from islet and pancreas blocks using the microtome

Blocks were kept cool until they were ready to be cut. Islet and pancreas sections were cut to a dept of 5µm and placed onto of a drop of water on specially treated glass slides (Menzel-Glaser®Superfrost®). These slides were left to dry on a heating block and placed in a 37ºC incubator over night.

2.2 ABO staining of paraffin embedded islets and pancreas sections with Mouse PAP

Paraffin wax was removed from slides by placing in xylene three times for 3 minutes, absolute ethanol twice for three minutes, 95% ethanol twice for three minutes, 70% ethanol twice for 3 minutes and rinsing in distilled water. The slides were then rinsed with PBS containing 0.05% tween. As controls, blood group A islets were stained using the B primary antibody and group B islets were stained using the A primary antibody. Dako peroxidase block (Dako, Sweden) was applied and slides were incubated for 5 minutes. Slides were rinsed with PBS buffer and incubated with serum block from animal where the secondary antibody is made to stop unspecific binding. In this case rabbit serum (Dako, Sweden) diluted 1:10 with Dako antibody diluent was applied for 15 minutes at room temperature. Primary antibody was then applied for 60 minutes in a humidity chamber in a 37ºC incubator:
Anti-A (Abcam, Cambridge, UK) antibody diluted 1:50

Anti-B (Dako, Sweden) antibody diluted 1:25

The slides were washed three times with PBS buffer and rabbit anti-mouse secondary antibody (Dako, Sweden) diluted 1:20 was applied for 30 minutes. Monoclonal mouse PAP (DakoCytomation, Sweden) diluted 1:125 was placed on slides for 30 minutes. Slides were rinsed three times in PBS buffer and developed using AEC/Chromogen (Dako, Sweden) by incubating in darkness for 15 minutes. Slides were counterstained with haematoxylin for 3 minutes and blued under running tap water for 5 minutes. Slides were then mounted and cover slipped.

2.3 ABO staining of paraffin embedded islets and pancreas sections using DakoCytomation EnVision+System-HRP (AEC) Kit

Paraffin wax was removed from slides and peroxidase block applied as described in section 2.2. The protocol was controlled in the same manner as before. Primary antibody was applied and incubated for 45 minutes in a humidity chamber in 37°C incubator:

Anti-A (Abcam, Cambridge, UK) antibody diluted 1:100

Anti-B (Dako, Sweden) antibody diluted 1:25

The slides were washed three times with PBS buffer and peroxidase labelled anti-mouse polymer from EnVision kit anti-mouse was applied for 30 minutes. Slides were developed, counterstained and mounted as described in section 2.2.
2.4 Staining paraffin embedded islet and pancreas sections for endothelial cells using ulex antibody

Slides were de-waxed and peroxidase block applied as described in section 2.2. The sections were rinsed in PBS buffer and ulex antibody (Bioscience, UK) diluted 1:500 was applied for one hour in 37°C incubator. Slides were washed three times with buffer and secondary antibody goat anti-ulex diluted 1:500 was applied for 30 minutes. Donkey anti-goat peroxidase (Dako, Sweden) diluted 1:50 was then applied for a further 30 minutes. Slides developed, counterstained and mounted as described in section 2.2.

Preparation and analysis of fresh islets

2.5 Isolation of islets

Islets were isolated using an automated digestion-filtration method followed by purification on a continuous density gradient in a refrigerated COBE 2991 centrifuge. Islet volume and purity were determined by microscopic sizing on a grid after staining with diphenylthiocarbazone. The purity of the islets ranged from 40 to 80%. The islet preparations were cultured in CMRL 1066 culture medium supplemented with 10nM nicotinamide, 10mM HEPES buffer, 0.25µg/ml fungizone, 50µg/ml gentamycin, 2mM L-glutamine, 10µg/ml Ciprofloxacin and 10% heat-inactivated human serum. The isolated islets were kept at 37°C in humidified air containing 5% CO₂.
2.6 Staining fresh islets with dithizone in order to assess purity

200µls of 1M NaOH and 100µls of 70% ETOH were added to a tube containing 5.8mg of dithizone. This was incubated at room temperature for 10 minutes and vortexed. After ensuring that all black powder has been dissolved, make this solution up to 5mls using 1 X PBS.

2.7 Staining fresh pig and human islets for ABO and collagen

Each bag of islets was divided into 50ml falcon tubes and allowed to sediment. Supernatant was discarded and the islets were washed twice with 1 X PBS by spinning at 1000rpm for one minute. Approximately 5000 islets were placed in each 2ml test tube (Ellerman), depending on how many antibodies were being tested. An irrelevant FITC antibody against mouse IgG (Dako, Sweden) and a negative control which contained only islets with no antibody added were also included. 10µls of undiluted Anti A (International Blood Group Reference Laboratory, IBGL, Bristol, UK) and Anti B (International Blood Group Reference Laboratory, IBGL, Bristol, UK) were added to the relevant tubes. 20µls of sub types of collagen I, II, III, IV, V, VII (Abcam, Cambridge, UK) were added to relevant tubes. In the case of collagen VI, 20µls, 40µls and 80µls of antibody was added as we had attached a fluorescent label so needed to determine correct concentration. Islets and antibodies were incubated for 30 minutes on an agitator in the cold room/on ice and washed twice with 1 X PBS. The COPAS and confocal microscope were used for analysis. The results were analysed in Cell Quest (BD Bioscience, Erembodegem, Belgium) as Mean Fluorescent Intensity (MFI). If samples were to be analysed on the confocal microscope the islets were fixed with 1% formaldehyde buffer and kept in the cold room protected from light.
The confocal microscope (Zeiss LSM 510 Meta, Jena, Germany) is equipped with an Axicert 200 microscope stand. Images were analysed using Imaris software.

2.8 Preparation of human hirudin plasma to incubate with islets

Blood was drawn from healthy blood donors into tubes containing the specific inhibitor of thrombin, recombinant hirudin (7ml blood substituted with 500µg of lepurudin), since we specifically wanted to investigate complement activation in both compatible and incompatible plasma in the absence of anticoagulants which would disturb the complement system. The blood samples were centrifuged at 3300 x g for 15 minutes and plasma harvested. This was used straight away and the remainder stored at –70°C. The donor blood samples were taken into EDTA tubes so these samples were passed through a hirudin buffered column to remove the EDTA.

2.9 Passing EDTA donor blood samples through buffer exchange column

Donor blood was centrifuged at 3220 x g for 5 minutes and plasma removed. Nap 25 column (Amersham Biosciences) was equilibrated with 25mls of CB++ buffer containing 70.4 µg/ml hirudin. 2.5mls of donor plasma was applied to the column. This was eluted with 3.5mls CB++ buffer containing 70.4 µg/ml hirudin and collected in 15ml tubes. The now hirudin plasma and the remaining EDTA-plasma were frozen at 70°C until analysis.
2.10 Double staining fresh islets for CD31-PE (endothelial cells) and ABO, C3c, IgG, IgM-FITC using compatible, incompatible and donor self plasma

The bag of islets was divided, sedimented and washed as described in section 2.7. Islets were divided evenly into various heparinised 2ml test tubes, (approximately 5000 islets in each), depending on how many antibodies were being tested. The test tubes used were coated with heparin to a surface concentration of 0.5µg/cm² which inhibits thrombin binding. An irrelevant FITC antibody against mouse IgG (Dako, Sweden) and a negative control which contained only islets with no antibody added were also included. 200µls of plasma (compatible, incompatible and donor) was added to each tube and incubated together for 30 minutes on agitator in 37ºC incubator. Islets were allowed to settle in tubes and as much plasma as possible was taken off. Islets were washed 3 times with 1 X PBS and appropriate antibodies: 10µls of undiluted Anti A, Anti-B, Anti-O (All from International Blood Group Reference Laboratory, IBGL, Bristol, UK), C3c, IgG, IgM, (All from Dako, Sweden) were added for 45 minutes on an agitator in the cold room. Islets were washed twice with 1 X PBS and analysed on the COPAS. Islets were fixed with 1% formaldehyde buffer and kept in the cold room protected from light before analysis on the confocal microscope.

2.11 Principle of Complex Object Parametric Analyser and Sorter (COPAS)

The COPAS is equipped with a quartz flow cell (1mm ID) and 670 nm red laser and 488/514 multi-line lasers. The emission of the red laser is collected by an optical detector and creates the extinction (EXT) parameter and the Time of Flight (TOF) parameter. The EXT parameter is a measure of particle optical density and TOF is a measure of a particle’s largest axis.
The emission of fluorochromes excited by the multi-line laser can be detected by three separate photon multiplier tubes with collection wavelengths separated by dichroic mirrors (510nm, 545nm, 580nm). The sample and sheath streams are diverted after analysis to a waste collector. Sorting is accomplished based upon criteria defined in the acquisition software by switching off the diverter for a set period of time to allow the particle to be collected. Sorted particles can be dispensed into a variety of vessels containing user selected buffers or media. The COPAS can accurately measure particles ranging from 40 to 500 µm in cross-sectional diameter. In this project one thousand islets were collected at a time. Data was collected as mean fluorescent intensity$^{32}$. 

Figure 2A showing the route particles flow through within the COPAS and where analysis takes place$^{33}$. 
2.12 Principle of Confocal Microscope (Zeiss 510 Meta Confocal, Carl Zeiss, Jena, Germany)

Laser scanning confocal microscopy, unlike conventional fluorescence microscopy, collects light from a single focal plane. It scans the specimen point-by-point, line-by-line and assembles the pixel information into a single image. An objective focuses an expanded light-beam to a small spot on the sample, at the focal plane of the objective lens. Reflected light from the illuminated volume of the specimen is collected by the objective and reflected by a beam splitter towards a pinhole arranged in front of the detector. In this case the pinhole is responsible for the confocal characteristic of the system. Information which does not originate from the focus level of the microscope objective is faded out by this arrangement. In contrast, light from the focal plane is focused on the detector pinhole and registered by the detector. The advantage of out-fading information from above or below the focal plane enables the confocal microscope to perform depth-dependent measurements: optical tomography becomes possible. A genuine 3D-image can be processed by confocal scanning of sequential levels.

Figure 2B showing the light path taken from the laser to the position of the islets and how the beams merge to form the 3-dimensional image we interpret.\textsuperscript{34}
Collagen immunohistochemistry work on paraffin embedded islet sections

2.13 Labelling collagen VI antibody with Alexa Fluor 488 monoclonal antibody labelling kit

A 1M solution of sodium bicarbonate was prepared by adding 1ml of deionised water to the vial of sodium bicarbonate provided in the kit. The collagen antibody was diluted to 1mg/ml and 1/10 volume of 1M sodium bicarbonate buffer was added. 100µls of this protein solution was added to the vial of reactive dye and the solution was incubated at room temperature for 1 hour. A spin column was prepared during this incubation time. 100µls of the incubated solution was added drop wise onto the centre of the column and allowed to be absorbed into gel bed. Column was placed into collection tube and centrifuged for 5 minutes at 1000 x g. The spin column containing the sodium azide dye was discarded. The collection tube now contained labelled protein in 100µls of PBS at pH 7.2. The degree of labelling was determined using a spectrophotometer. This antibody was used to stain fresh islets and paraffin wax embedded islets for collagen VI. Analysis took place on the COPAS and confocal microscope.

2.14 Staining paraffin embedded islet and pancreas sections for collagen type VI
(which we attached fluorescent label to using alexa fluor 488 protein labelling kit)

Paraffin wax was removed, slides were washed and peroxidase block applied as described in section 2.2. Pancreas sections were used as positive collagen controls. Collagen VI antibody (Abcam, Cambridge, UK) was applied to slides at concentrations of 5, 10 and 20µls and incubated for 30 minutes in the darkness.
Slides were counterstained and blued as described in section 2.2. Slides were mounted using fluorescent mounting media and cover slipped. Analysis took place on microscope using the fluorescent settings.

2.15 Staining paraffin embedded islet and pancreas sections for collagen using mallory trichrome staining kit (Bio Optica, Milan, Italy)

Sections were brought to distilled water. Five drops of carbolfuchsin according to Ziehl and seven drops of distilled water were placed on slides and allowed to act for 10 minutes. Slides were rinsed and five drops of distilled water, three drop of acid buffer and five drops of formalin solution were applied to the slides for 2 minutes. Slides were washed quickly in distilled water and ten drops of phosphomolibdic acid solution was applied for 5 minutes. Slides were drained and ten drops of polychrome solution according to Mallory was added for 5 minutes. Slides were washed in distilled water, dehydrated rapidly in ascending alcohols and cleared in xylene. The slides were mounted in mountex and cover slipped.

2.16 Staining paraffin embedded islet and pancreas sections for collagen using Weigert Van Gieson stain

Slides were brought to distilled water. Weigerts haematoxylin was added for 5 minutes and slides were rinsed in tap water and differentiated in spirit. Slides were then blued in tap water for 5 minutes and Van Gieson was added for 95 seconds. Slides were washed again in tap water and dehydrated in ascending alcohols. Sections were cleared in xylene and mounted using mountex.
3.0 Results

3.1 Immunohistochemistry Results

Paraffin embedded sections were stained for ABO using two optimised methods: A Dako Envision method and a mouse PAP method where a secondary antibody was used. Arrows point at isles within pancreas.

Figure 3.1A shows an AB pancreas stained for A using the PAP method. Viewed X 60
Figure 3.1B shows an AB pancreas stained for A using the Envision method. Viewed X 20.
Figure 3.1C shows an AB pancreas with islet within stained for B using the Envision kit. Viewed X 60.

Figure 3.1D shows AB islets stained for A using the PAP method. Viewed X 20.
Figure 3.1E shows AB islets stained for B using the Envision method. Viewed X 20.
Figure 3.1F shows A islet stained for A using the PAP method. Viewed X 40.

Figure 3.1G shows A islet stained for A using the Envision method. Viewed X 40.

Figure 3.1H shows AB islets stained for B using the PAP method. Viewed X 40.

Figure 3.1I shows AB islets stained for B using the Envision method. Viewed X 40.
Figure 3.1J

Figure 3.1J Negative control. A islets stained for B. Viewed X 20.

Paraffin sections were also stained for endothelial cells using CD31 and Ulex lectin. Endothelial cells express the ABO antigens. Staining with CD31 proved unsuccessful. The ulex lectin binds to the H part of the ABO chain thereby indirectly detecting endothelial cells (red colour). Arrow points to islet within pancreas.

Figure 3.1K                                                  Figure 3.1L

Figure 3.1K Shows pancreas section stained using ulex lectin. Islet can be seen within. Figure 3.1L Islet section stained using ulex europaeus lectin. Peri-capsule seems to be strongly stained. Perhaps this is remaining exocrine parts.
3.2 Staining fresh islets with dithizone

Collagen is present in the exocrine part of the pancreas. If these exocrine parts are still attached to the islets after isolation then this may contribute to islet rejection. Dithizone stains zinc present in the islets but does not stain exocrine fragments. The washing steps performed during islet preparation for the COPAS may remove some of the loosely bound exocrine fragments.

Figure 3.2A

Figure 3.2B

Figure 3.2A: Islets stained for zinc using dithizone before washing steps. Exocrine fragments (lighter yellow colour) seem to bind to and overlap some of the brown staining islets.

Figure 3.2B: Islets stained for zinc again after the washing step. Exocrine fragments seem fewer and don’t seem to be bound to the islets so much.
3.3 Confocal Results

Fresh islets were stained using Anti-A, B and O-FITC conjugated antibodies. The confocal provided 3-dimensional pictures of the staining pattern on the islet. This pattern varied between islets incubated in autologous, compatible and incompatible plasma.

Figure 3.3A
Figure 3.3B

Figure 3.3A: A islet incubated in compatible plasma and stained for A
Figure 3.3B: A islet incubated in incompatible plasma and stained for A

Figure 3.3C
Figure 3.3D

Figure 3.3C: B islet incubated in compatible plasma and stained for B.
Figure 3.3D: B islet incubated in incompatible plasma and stained for B.
O islets are compatible with all blood types so they were incubated with compatible A plasma and donor plasma.

Figure 3.3E: O islets incubated in compatible A plasma and stained for O.
Figure 3.3F: O islets incubated in autologous plasma and stained for O.
Figure 3.3G: Negative control A islet stained for B

Fresh islets were also stained for IgG, IgM and C3c-FITC after incubation with autologous, compatible and incompatible plasma. The staining pattern varied.

Figure 3.3H: O islets incubated with autologous plasma and stained for IgG.
Figure 3.3I: A islets incubated with A plasma (compatible) and stained for IgG.
Figure 3.3J: A islets incubated with B plasma (incompatible) and stained for IgG.
Figure 3.3K: B islets incubated with B plasma (compatible) and stained for IgG.

Figure 3.3L: B islets incubated with A plasma (incompatible) and stained for IgG.

Figure 3.3M: O islets incubated with autologous plasma and stained for IgM.

Figure 3.3N: A islets incubated with A plasma (compatible) and stained for IgM.

Figure 3.3O: A islets incubated with B plasma (incompatible) and stained for IgM.
Figure 3.3P: B islets incubated with B plasma (compatible) and stained for IgM.

Figure 3.3Q: B islets incubated with A plasma (incompatible) and stained for IgM.

Figure 3.3R: Islets incubated in donor autologous plasma and stained for C3c.

Figure 3.3S: Islets incubated in compatible plasma and stained for C3c.

Figure 3.3T: Islets incubated in incompatible plasma and stained for C3c.

Figure 3.3U: Irrelevant control showing no positivity
3.4 Immunohistochemistry staining for collagen

Different stains were used to stain pancreas and islet sections for collagen. It is thought that collagen may play a role in the destruction of transplanted islets. Collagen VI is known to be the predominant type present in islets of langerhans. Weirgerts van Geison and Mallory Trichrome were both used as a general collagen stain. Mallory trichrome gave the best results staining collagen a light blue colour. A fluorescent label was also attached to a collagen VI antibody and paraffin sections were stained and examined under fluorescent microscope.

Figure 3.4A: Pancreas section stained using mallory trichrome. Arrow pointing at islet within pancreas. Viewed X 20

Figure 3.4B: Islet showing peri-capsule of collagen. Viewed X 60

Figure 3.4C: Islet showing some collagen. Viewed X 20
Figure 3.4D: Pancreas stained with Abcam immunohistochemistry Col VI. Arrow pointing at islet within. Viewed X 20

Figure 3.4E: Same pancreas section viewed X 60. Islet staining strongly with Collagen VI (red colour).

Figure 3.4F: A islet stained with collagen VI. Viewed X 40.

Figure 3.4G: Pancreas section viewed under normal light that has been stained with collagen VI with fluorescent label attached. Viewed X 40.

Figure 3.4H: Same pancreas section viewed under UV light. Viewed X 40.
3.5 COPAS analysis

These are examples of dot plots from the COPAS. In this case blood group A islets have been analysed.

Figure 3.5A: showing just islets

Figure 3.5B: A islets stained for A

The arrow points to the gated area that is used for analysis. This area contains pure islets.

Figure 3.5C: Negative control, A islets stained for B.
Bar charts were made using the information gathered from analysis done on the gated area from the dot plots described above. Cell Quest Pro (BD Bioscience, Erembodegem, Belgium) was the computer programme used for analysis.

![Bar chart of ABO expression](image)

**Figure 3.5D:** Bar chart of ABO expression on islets alone (blue) and on islets stained for blood group antigens (red) plotted against percentage of total islets expressing blood group positivity.
Figure 3.5E: Bar chart of group A islets incubated with compatible A plasma (blue) and incompatible B plasma (red) and stained for A, B, C3c, IgG and IgM plotted against the mean fluorescent intensity.

Islets alone can have some outer fluorescence so this is also included. An irrelevant containing mouse IgG is used to out rule non-specific binding.
Figure 3.5F: Bar chart of O islets incubated with autologous plasma (red) and compatible plasma (blue) and stained for C3c, IgG and IgM and plotted against mean fluorescent intensity. Islets alone were included as a control.
4.0 Discussion

The number of type 1 diabetics in the world is continually increasing however the supply of donor organs remains quite low. The question from the onset of this project was is it possible to transplant islet of Langerhans cells across the ABO barrier because of this huge demand for organs. When we consider ABO staining in both the immunohistochemistry pictures and the COPAS with confocal analysis the answer appears to be no as exocrine parts still remain on the islets containing moderate amounts of ABO epitopes. Even islets not covered by exocrine fragments express a small amount of ABO positivity on the blood vessels that are within the cells themselves. The Dako EnVision method proved to give a more intense and amplified staining pattern than the mouse PAP method. Both Envision and PAP methods were optimised but Anti-B did not work well with PAP even when a very low dilution of the antibody was used (figure 3.1H). When pancreas sections were stained for blood group antigens, the islets within the pancreas didn’t stain positively. However, when the isolated islet sections were stained for blood group antigens positivity was seen. Perhaps this is a result of exocrine parts remaining on the islets even after isolation or maybe it’s a result of the collagenase used during isolation. Use of collagenase during isolation may have led to generation of neo-epitopes, which may have been the target for the antibodies. As collagenase itself is a bacterial by-product, there may be remnants of the enzyme or its products left on the islets that could serve as targets for the antibodies. When ulex europaeus lectin was used, this is a marker for vascular endothelium, the islet stains but not as intensely as the rest of the exocrine parts of the pancreas. When isolated islets were stained with ulex, the peri-capsule ring around the outside of the islet stains more positively than the rest of the islet. This could be some exocrine parts remaining around the islet edge. It is a fair assumption to make that exocrine parts remain after isolation and that the washing steps done during islet
preparation for the COPAS may remove some of these parts. Figures 3.2A and 3.2B shows fresh islets stained with dithizone before and after washing. Dithizone stains zinc present in the islets a dark brown colour. It does not stain the exocrine parts as there is no zinc present. Before washing there are lots of exocrine parts attached to and covering the islets. After washing the islets with PBS there are fewer islets covered and although the overall picture may show some exocrine parts still attached, it is a lot less than before the washing step. When fresh islets were stained with anti-ABO FITC and analysed by the COPAS, the ABO expression was not as high as the immunohistochemistry pictures may suggest. This washing step may account for this. Also the islets had been suspended in culture media which also may have removed some exocrine parts. The gated part of the graph that is used for analysis on the COPAS contains only islets. The COPAS result of ABO expression therefore may be more accurate than the immunohistochemistry pictures as the true islet is being analysed with exocrine parts having been washed away. It should be remembered however that the islets transplanted to diabetic patients are not washed in such an intensive manner so the recipient is receiving exocrine parts as well as islets. These exocrine parts are rich in ABO antigens as well as other substances which is enough to activate the complement cascade and lead to islet destruction. In the confocal pictures the ABO expression is less than in the immunohistochemistry sections and the staining pattern resembles endothelial expression as well as ABO expression. This can be seen particularly well in figures 3.3E. The endothelial cells line blood vessels and are the ones expressing the ABO antigens so even islets that don’t contain exocrine parts have a minimal amount of ABO epitopes on the endothelial cells lining the vessels that run within the islet. 5-10% of an islet contains blood vessels which are all lined by endothelial cells. COPAS results shows a similar result for ABO expression. Figure 3.5D shows a result of 6% for ABO expression on the fresh islets that I investigated throughout this project.
The exocrine part of the pancreas contains a substantial amount of collagen. This has been demonstrated using mallory trichrome staining technique (figure 3.4A). Collagen VI has been shown to be the main collagen subtype present within islets\textsuperscript{22}. Staining using Abcam collagen VI antibody shows a positive staining result for the islets within the pancreas and a peri-capsular staining pattern for the islet sections (figure 3.4B). A fluorescent collagen VI antibody also showed a similar staining pattern. It is quite possible that because isolated islets have exocrine parts attached that are rich is collagen, a destructive process could be stimulated by activation of natural antibodies we as humans have against collagen\textsuperscript{38}. The recipient would recognise this collagen as a foreign body and the complement system would be activated resulting in rejection and destruction of the transplanted islets.

The second part of the project was comparing ABO, IgG, IgM and C3c expression between islets incubated with compatible, incompatible and self donor plasma. When islets were incubated with incompatible plasma the expression of ABO antigens decreased. Perhaps complement activation destroys some of the ABO epitopes resulting in a lower antigen expression. IgG, IgM and C3c rise when the islets are incubated with incompatible plasma so some or all of these three are binding to or masking the ABO epitopes resulting in ABO expression appearing decreased. It is interesting that when islets were incubated with compatible plasma IgG, IgM and C3c were still activated. It should be noted that this is the case in a diabetic recipient. Islets are ABO matched yet after transplantation a diabetic only has 20% functional islet capacity to that of a non-diabetic as a result of this complement activation and destruction. This may be a result of natural antibodies to the collagen or maybe some other substance present on the exocrine portions. When islets were incubated with donors own plasma the blood group expression actually decreased when compared to islets incubated with compatible plasma (figure 3.3F). In the case of
this project, O islets were incubated with A plasma and with self plasma. It was not possible to incubate O islets in incompatible plasma as blood group O is the universal donor. The decreased O expression when donor islets are incubated with donor plasma may be due to the manipulation carried out on the donor blood sample when placing it through the hirudin column. The donor blood sample was taken into a tube containing EDTA so this anticoagulant needed to be removed and replaced with hirudin. The CB++ buffer used in the column contained 5M barbiturate which has been found out to be toxic to islets. Barbiturate competitively binds to reactive sites on or within the pancreatic B-cells. It also has a damaging effect upon ATP-K+ channels and voltage-activated channels present in the plasma membrane of insulin-secreting cells. This barbiturate could have damaged the ABO antigens resulting in inability to bind the antibody. However, because of lack of islet supply in this project, this experiment was only carried out three times using three different O islet donors incubated with plasma from three different compatible blood group A people and incubated with donor autologous plasma. Further testing using group A and B islets would give us a better picture of what is really happy as incompatibility testing could also be done which is not the case with group O islet donors. Ideally donor blood samples should be taken into hirudin tubes from the beginning so that there would be no need to pass the blood through and exchange column. In the future if a column is to be used PBS buffer containing the appropriate ions should be the buffer of choice rather then CB++ buffer which contains barbiturate. C3c levels rise significantly when islets are incubated with incompatible plasma (figure 3.3T). An IgG and IgM rise soon follows. This is not the case when islets are incubated in autologous plasma which would make sense as the islets should not recognise autologous plasma as something foreign therefore little of no complement activation should take place. Perhaps the barbiturate is only capable of damaging the ABO antigens as the IgG and IgM binding
sites are in general more robust so can withstand the damaging effects of barbiturate better. One study by Titus et al concluded that there was evidence of complement binding by the classical pathway along with IgG binding during human islet-allogeneic blood interaction. This study also believes that contact with allogeneic blood is the initiating event that leads to the immediate islet destruction. C1q was found deposited on the islets which further suggests that the classical pathway of complement activation is involved. Involvement of the lectin pathway and the alternative pathway cannot be excluded. Titus et al found small amounts of IgG and IgM deposited on the islets but they did not believe that this was solely responsible for the complement activation as the isotypes of IgG vary in their capacity to activate this cascade. It is possible that there may be auto antibodies that bind to self-proteins or antibodies reacting to self neo-epitopes. It has been shown that isolated islets lack regulatory proteins that control complement activation. This too could play a part in the spontaneous activation that occurs when transplanted islets come in contact with recipient’s blood.

There is a fine line between making the islets as pure as possible and over digestion with collagenase which may cause damage to the islets. The ultimate goal is to transplant a sufficient amount of functional and compatible islets into the liver of the diabetes type 1 patient so that engraftment can occur. The in growing blood vessels rarely enter the clusters of endocrine cells in the grafts but remain located in the graft stroma so transplanted islets usually have low oxygen levels. The glucose levels in the portal blood are higher than those in other sites. This exerts a greater stress on the transplanted islets and often exhausts them before they have readily engrafted. The process of islet isolation and culture destroys the islet microvasculature, forcing the islets to depend on passive diffusion immediately after intrahepatic islet transplantation in order to obtain the necessary nutrients and an adequate oxygen supply. It should be remembered that the
actual islets that a recipient receives have exocrine parts attached containing a considerable
amount of both ABO and collagen antigen expression. As a result of this the majority of
the transplanted islets don’t engraft. This could be part of the reason for the complement
activation and islet destruction upon transplantation.

To conclude, it is not exactly known what is present on the islet surface that is
leading to activation of the complement cascade and islet destruction. The findings of my
project would point towards ABO antigens and collagen being two of the main players in
this destructive process. A suggestion for the future may be to incubate fresh islets in
compatible diabetic blood to see if this affects C3c, IgG and IgM levels more or less than
when islets are incubated in compatible blood of a non-diabetic. We are getting closer to
answering the question of why islets are destroyed once they come in contact with
recipient’s blood. Perhaps one day in the not so distant future one donor pancreas will
sufficiently provide enough islets to engraft and provide insulin independence within the
diabetic recipient.
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