Sertoli Cells and Complement Inhibitors: A Possible Mechanism to Increase Pancreatic Islet Viability

Rachel L Washburn¹, Gurvinder Kaur² and Jannette M Dufour*³

¹Department of Immunology and Infectious Disease, Texas Tech University Health Sciences Center, USA
²Department of Medical Education, Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, USA
³Department of Cell Biology and Biochemistry, Immunology and Infectious Disease and Medical Education, Texas Tech University Health Sciences Center, USA

Abstract

Type I Diabetes Mellitus (TIDM) is an autoimmune disease in which the immune system attacks and destroys the insulin producing β cells of the pancreatic islets. Insulin is an essential hormone released in response to elevated blood glucose levels, allowing the uptake and utilization of glucose by muscle and adipose tissue. The establishment of an immune privileged environment by Sertoli cells (SCs) through several mechanisms, including inhibition of complement mediated cell lysis by production of complement inhibitor proteins, may offer a unique alternative to prolong graft survival. Further understanding of how SCs use complement inhibition to incite immune protection could be translated into increasing the viability of pancreatic islet transplants.

Keywords: Islet transplantation; Hyperacute rejection; Type I diabetes; Complement; Sertoli cells

Introduction

Type I Diabetes Mellitus (TIDM) is an autoimmune disease in which the immune system attacks and destroys the insulin producing β cells of the pancreatic islets. Insulin is an essential hormone released in response to elevated blood glucose levels, allowing the uptake and utilization of glucose by muscle and adipose tissue. Patients with TIDM require regular administration of exogenous insulin to prevent life threatening effects of chronic hyperglycemia like diabetic ketoacidosis [1]. At this time, the preferred treatment of TIDM is lifelong insulin replacement therapy, in which insulin is administered by injection, or continuously through an insulin pump. Yet, this treatment is costly and achievement of normoglycemia is very difficult. Even with patient treatment compliance, which is difficult to achieve, only 37% of patients with TIDM are able to adequately control blood glucose levels through exogenous insulin administration [2].

Islet Transplantation Method for TIDM

Islet transplantation is a promising alternative treatment for TIDM, as the transplanted β cells would be able to produce endogenous insulin in direct response to the patients’ blood glucose levels, offering a virtual cure for TIDM. However, in order for the transplanted islets to remain viable, patients undergoing this procedure are required to take chronic immunosuppressive drugs for the rest of their life in order to limit transplant rejection. These drugs have been linked to an increased risk of developing infections as they suppress the immune system’s ability to fight pathogens [3]. They have also been linked to increased malignancies and cardiovascular disease and have even been shown to be toxic to the recipient’s organs and the grafted tissue [3]. Even with immunosuppressive therapy, the islets are rejected and only about 10% of islet transplant patients remain in exogenous insulin free state after five years post transplantation [4]. Additionally, there is a shortage of human organs available for transplantation, while the need for transplants for diabetes patients has increased [5]. Use of pancreatic islets isolated from pigs would solve the issue of organ shortage. For instance, neonatal pig islets transplanted into pancreatectomized rhesus macaques survived for over 100 days and resulted in insulin independence after the recipients were treated with a CD28 to CD154 co-stimulation blockade. However, the pig islets transplanted into non-immunosuppressed macaques were rapidly rejected [6,7]. Overall, this suggests that the immune response to xenografts is even more robust than the response to allografts and therefore, pig islets are quickly rejected by...
the patient’s immune system initially due to hyper acute rejection. If any cells or tissue survive hyper acute rejection, delayed rejection occurs through an activated adaptive mechanism involving B cells and T cells. This mini review will focus on hyperacute complement-mediated rejection.

**Hyperacute Rejection**

Hyperacute rejection occurs when preformed antibodies bind to foreign carbohydrates or surface antigens expressed by the transplanted tissue. This activates the complement cascade and results in cellular lysis. Additionally, anaphylatoxins are released promoting inflammation and chemotaxis. The most well characterized xenograft is Gal-α1,3Gal-β1,4GlcNAc-R, more commonly known as α-Gal [8]. α-Gal is a cell surface carbohydrate produced by the enzyme galactosyltransferase expressed in nearly all animals, excluding humans and old world monkeys. α-Gal has a similar structure to bacterial surface markers from colonic bacteria that induce the human immune system to create Xenoreactive Natural Antibodies (XNA), which can bind to and target α-Gal-related antigens [8]. These XNA antibodies, of the IgG and IgM moieties, readily bind to α-Gal when it is present in the body. Thus, xenografts are hyper acutely rejected by a humoral immune process, with the complement system playing a major role [9-12].

**Complement Inhibitor Proteins Expressed by Sertoli Cells**

The complement system is a cascade of protease enzymes that is activated by the binding of antibodies or pathogen antigens (Figure 1).

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**Table 1:** Complement inhibitor proteins expressed by Sertoli cells.

<table>
<thead>
<tr>
<th>Inhibitor Protein</th>
<th>Alternate Designations</th>
<th>Pathway Inhibited</th>
<th>Inhibition Point</th>
<th>Function in Complement Cascade Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 Inhibitor</td>
<td>C1 INH, Serpin G1</td>
<td>Classical</td>
<td>C1q,r,s</td>
<td>Binds both C1r and C1s to dissociate them from C1q,r,s complex</td>
</tr>
<tr>
<td>Membrane Cofactor Protein</td>
<td>MCP, CD46</td>
<td>Classical, Lectin, Alternative</td>
<td>C3 and C5 convertases</td>
<td>Cofactor for Factor I, deactivates C3b and C4b</td>
</tr>
<tr>
<td>Decay-Accelerating Factor</td>
<td>DAF, CD55</td>
<td>Classical, Lectin, Alternative</td>
<td>C3 and C5 convertases</td>
<td>Dissociates C3 and C5 convertases by displacing C2a from C4b and Bb from C3b</td>
</tr>
<tr>
<td>Clusterin</td>
<td>CLU, Sulphated Glycoprotein-2, SGP-2, Apolipoprotein J, ApoJ</td>
<td>Terminal</td>
<td>MAC</td>
<td>Binds terminal complement proteins C5-C8, preventing their insertion into target membrane; when bound with vitronectin, causes MAC to become soluble</td>
</tr>
<tr>
<td>CD59</td>
<td>Protectin</td>
<td>Terminal</td>
<td>MAC</td>
<td>Inhibits C8, blocks C9 from binding C5b8 complex</td>
</tr>
</tbody>
</table>

1Factor I inhibits the classical, alternative and lectin pathways of complement activation by cleaving C3b and C4b
2Vitronectin inhibits C8 binding to C5b7, and when bound to clusterin causes MAC to become soluble
Activation of the complement cascade results in formation of the C3 and C5 convertases and culminates with the formation and insertion of an intermembrane pore called the Membrane Attack Complex (MAC), which functions to lyse the target cell [13]. Complement also has opsonization functions, where C3 and C4 components coat the target cells and mark them for destruction by leukocytes. Xenografts of porcine pancreatic islets are sensitive to humoral immunity-related hyperacute graft rejection. The complement cascade has been shown to kill neonatal porcine islets both in the presence and absence of antibodies, indicating porcine islets are killed by both the classical and alternative pathways of complement mediated cell lysis, respectively [10-12]. There are several complement regulatory proteins that can inhibit the complement cascade and protect tissue from complement-mediated cell lysis (Table 1) [13,14]. These regulatory protein functions to inhibit different stages of the complement cascade (Figure 1). Membrane Cofactor Protein (MCP, CD46) and Decay Accelerating Factor (DAF, CD55) each inhibit the formation C3 convertase in both the alternative and classical pathways. Inhibition of later steps in complement mediated cellular lysis occur through CD59 (protectin) and C5 convertases and culminates with the formation and insertion of an intermembrane pore called the Membrane Attack Complex (MAC), which functions to lyse the target cell. Protection of the auto-immune privileged enterocytes, which produce several complement regulatory proteins, is necessary as the advanced germ cells do not appear until puberty, which is long after peripheral immune tolerance has been established. This immune privileged environment is created both by maintaining the blood testis barrier and through secretion of immunosuppressive and immunoregulatory factors [17]. Whereas transplanted islet cells are hyperacutely destroyed by factors such as the complement cascade, ectopically transplanted SCs enjoy the same immune privilege as testicular-located SCs, implying that immune protection can be achieved through SC-produced immunoregulatory factors and complement inhibitors [11,18]. Additional studies have shown that when pancreatic islets are coupled with SC grafts, the transplants survive even without the use of harsh immunosuppressive therapies [18]. SCs have been shown to inhibit human natural antibody mediated lysis and produce several complement regulatory proteins [11,19-21]. Using an in vitro humoral assay, Neonatal Porcine Sertoli Cells (NPSCs) were found to have increased survival when exposed to human serum containing complement as compared to Porcine Aortic Endothelial Cells (PAECs), with NPSC survival rate increasing to over 160%, and PAEC survival rate declined to about 30% [19]. Further analysis demonstrated NPSCs were positive for the α-Gal epitope and deposition of human IgM and IgG antibodies after exposure to human serum [19-21]. NPSCs were further analyzed to
determine if either the alternative or classical complement pathways were activated. The classical pathway protein C4 was observed to be deposited on the membrane, thus inhibiting cell lysis. Additionally, NPSCs and Neonatal Porcine Islets (NPIs) were cultured in human serum containing complement and survival was examined with an in vitro cytotoxic assay. While NPSCs enjoyed an increase in survival, there was a decrease in survival of the NPIs both when exposed to human antibodies with complement and complement alone, suggesting NPIs are killed through both the alternative and classical pathways [11,12]. Furthermore, NPSCs and NPIs were transplanted into naive Lewis rats [11]. While NPI xenografts were rejected completely by day nine, NPSC grafts survived the 20 day duration of the study even though the rats were not treated with immune suppressing drugs. Analysis of the grafts found C3 deposition in both NPI and NPSC xenografts. Interestingly, the NPI grafts showed extensive MAC deposition. NPSC grafts, on the hand, showed no MAC deposition [11]. SCs express several complement inhibitors including: C1 inhibitor, MCP, DAF, CD59, and clusterin (Figure 1 and Table 1) [11,20,22]. NPSCs were found to express significantly elevated levels of both MCP and DAF as compared to NPI cells [11]. In a preliminary attempt to analyze the importance of MCP and DAF on NPSC survival of human antibody complement-mediated cell lysis, NPSCs were transduced with MCP or DAF shRNA to knock down their expression. After 24 h, these transduced NPSCs were cultured with human serum and complement and cell viability was assessed by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay. While NPSCs enjoyed an increase in survival, there was a decrease in survival of the NPIs both when exposed to human antibodies with complement and complement alone, suggesting NPIs are killed through both the alternative and classical pathways [11,12].

Conclusion

The establishment of an immune privileged environment by SCs through several mechanisms, including inhibition of complement-mediated cell lysis by production of complement inhibitor proteins, may offer a unique alternative to prolong graft survival. Further understanding of how SCs use complement inhibition to incite immune protection could be translated into increasing the viability of pancreatic islet transplants. In this manner, patients would not be required to take harsh immunosuppressive drugs in order to prevent islet graft rejection. Furthermore, porcine islets may become a more viable islet transplant option, resolving the issue of organ donor shortages and making pancreatic islet transplants a more accessible treatment to achieve normoglycemia for TIDM patients.

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References

19. Dufour JM, Hamilton M, Rajotte RV, Korbutt GS. Neonatal porcine

