Annals of Diabetes Research

Review Article
Published: 24 Feb, 2020

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

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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

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Received Date: *23 Jan 2020*
Accepted Date: *21 Feb 2020*
Published Date: *24 Feb 2020*

Citation:
Washburn RL, Kaur G, Dufour JM. Sertoli Cells and Complement Inhibitors: A Possible Mechanism to Increase Pancreatic Islet Viability. Ann Diabetes Res. 2020; 4(1): 1013.

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

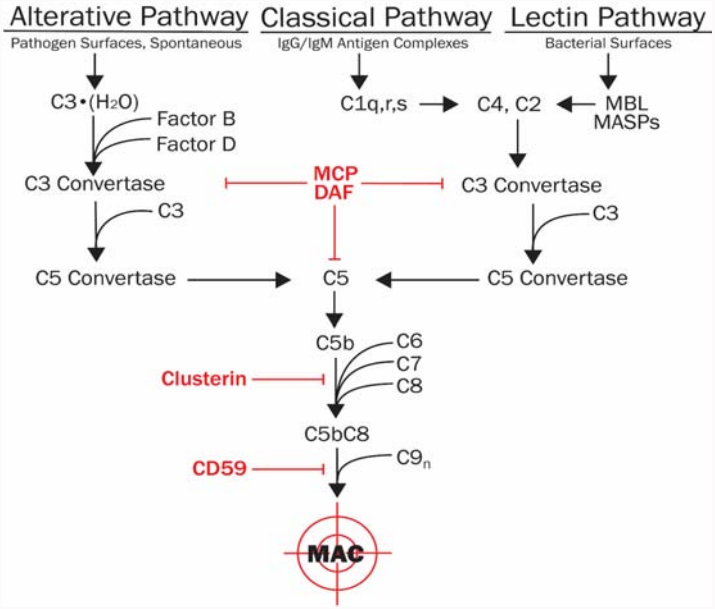

Figure 1: A basic overview of the complement cascade. The complement cascade can be activated by three different pathways: the Alternative Pathway (AP), Classical Pathway (CP), and Lectin Pathway (LP). AP is activated when soluble C3 binds to a pathogen's surface. Once bound, factor B binds and cleaves C3, forming C3bB. Factor D then cleaves B, forming the AP C3 convertase, C3bBb. More C3 will bind and become cleaved by C3 convertase to form C3bBbC3b, the AP C5 convertase. CP and LP form the same C3 convertase, but are activated by different targets. CP is activated upon antibody binding, usually IgG and IgM, to the C1q,r,s complex, which is bound to the target surface. This C1 antibody complex then cleaves C4 and C2. LP is activated by bacterial surface lectins, recruiting Mannose Binding Lectins (MBL) and Mannan-Associated Serine Proteases (MASPs) to bind, which go on to cleave C4 and C2. At this stage, both CP and LP form C4bC2a, the CP/LP C3 convertase, which bind and cleave C3 forming C4bC2aC3b, the CP/LP C5 convertase. Both C5 convertases binds C5 and cleave it to its active form, C5b. Once C5b is bound to the pathogen surface, it then binds C6, C7, and C8 which forms the C5bC8 complex that goes on to recruit multiple C9 proteins, forming the Membrane Attack Complex (MAC), the culminating step in complement-mediated cell lysis. Additionally, C3b coating of target surfaces by all pathways promotes opsonization and phagocytosis of the target cells. The complement pathway can be inhibited at four main steps: integrity of the C1 complex, the formation of C3 convertase, the formation of C5 convertase, and the assembly of the MAC. C1 inhibitor binds C1r,s and causes dissociation of C1q,r,s. Membrane Cofactor Protein (MCP, CD46) cleaves C3b and C4b further, which prevents assembly of both C3 and C5 convertases. Decay-Accelerating Factor (DAF, CD55) blocks C3b and C4b binding, thus preventing the formation of the C3 and C5 convertases. Clusterin binds to the terminal proteins, mainly C7, and prevents them from assembling C5bC8. CD59 (protectin) prevents recruitment of the C9 pore-forming proteins to C5bC8, effectively inhibiting MAC formation.

Table 1: Complement inhibitor proteins expressed by Sertoli cells.

Inhibitor Protein	Alternate Designations	Pathway Inhibited	Inhibition Point	Function in Complement Cascade Inhibition
C1 Inhibitor	C1 INH, Serpin G1	Classical	C1q,r,q	Binds both C1r and C1s to dissociate them from C1q,r,s complex
Membrane Cofactor Protein	MCP, CD46	Classical, Lectin, Alternative	C3 and C5 convertases	Cofactor for Factor I[1], cleaves C3b and C4b
Decay-Accelerating Factor	DAF, CD55	Classical, Lectin, Alternative	C3 and C5 convertases	Dissociates C3 and C5 convertases by displacing C2a from C4b and Bb from C3b
Clusterin	CLU, Sulphated Glycoprotein-2, SGP-2, Apolipoprotein J, ApoJ	Terminal	MAC	Binds terminal complement proteins C6-C8, preventing their insertion into target membrane; when bound with vitronectin[2], causes MAC to become soluble
CD59	Protectin	Terminal	MAC	Inhibits C8, blocks C9 from binding C5b8 complex

[1]Factor I inhibits the classical, alternative and lectin pathways of complement activation by cleaving C3b and C4b
[2]Vitronectin inhibits C8 binding to C5b7, and when bound to clusterin causes MAC to become soluble

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 xenoantigen 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).

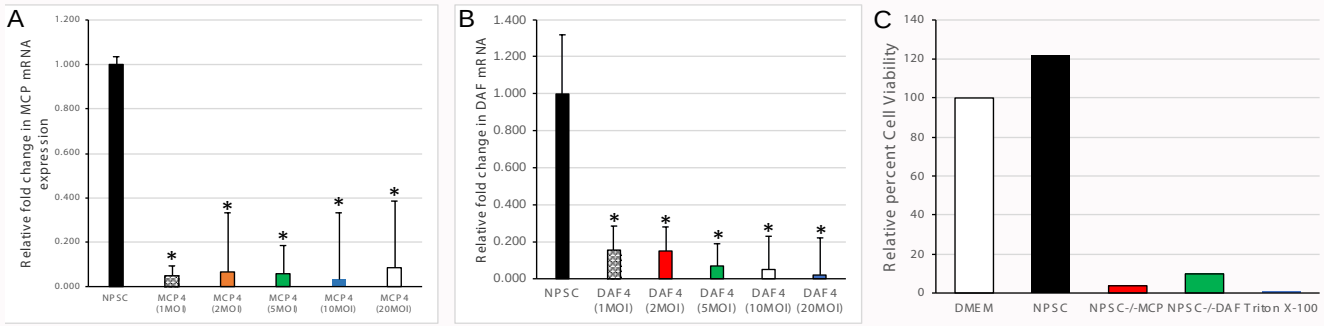

Figure 2: Complement mediated cell lysis. A and B) To optimize the transduction efficiency, MCP and DAF shRNAs were purchased from Origene Technologies Inc. (Rockville, MD). NPSCs, isolated from 1 to 3days old piglets, were cultured overnight and then transduced with MCP (A) or DAF (B) shRNA at 1, 2, 5, 10 and 20 Multiplicity of Infection (MOI). After 24 h, RNA was collected and isolated from transduced NPSCs. qPCR for MCP (A) or DAF (B) was performed using mRNA isolated from transduced NPSCs. 18s was used as an endogenous control and the fold change for the gene of interest was calculated relative to the level in the reference sample (Non-transduced NPSCs (black bar)). One-way ANOVA followed by a Tukey's post hoc test (*$p \leq 0.05$) was used to determine significant differences as compared to the reference sample. The mRNA expression of MCP (A) and DAF (B) in transduced NPSC were significantly reduced at all the MOIs tested. However, MCP and DAF at 1MOI were optimal in inhibiting the expression without any cell toxicity. Based on this information, MCP or DAF shRNAs at 1MOI were used to knock down the expression in NPSCs for the human antibody/complement-mediated lysis experiment. C) To determine the role of MCP or DAF in complement mediated cell lysis of NPSCs, NPSCs (n=1) were transduced with MCP or DAF shRNA at 1 MOI. After 24 h these cells were exposed to human serum plus complement and cell viability was assessed by MTT assay. Non-transduced NPSCs were used as control (black bar). Viability of non-transduced cells exposed to media alone (white bar) was normalized to 100% and the relative percent cell viability of NPSCs transduced with MCP (green bar) or DAF (red bar) shRNA was graphed. NPSCs cultured in triton-X 100 were used as positive controls for cell lysis.

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 proteins function 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 clusterin. CD59 blocks the C9 proteins from binding to the C5b8 complex, and thus inhibits MAC formation. Clusterin binds to the C5b9 complex, inactivating MAC cellular lysis by increasing the complex's solubility [14]. Transplantation of tissue expressing these complement regulatory proteins could be used to prolong xenograft survival. Transgenic pigs have been generated to address the issue of hyperacute complement mediated xenograft rejection. Two approaches are under investigation: elimination of reactive surface antigens and induced expression of human complement inhibitors. When α-Gal was knocked out in porcine islets, human antibody binding was significantly reduced *in vitro*. However, when these same islets were transplanted into diabetic rhesus monkeys, a hyperacute inflammatory reaction occurred when immunosuppressive therapy was not utilized [15]. It has now been shown that, while α-Gal is the predominate xenoantigen expressed by pigs; porcine islets express other xenoantigens important for graft rejection. This suggests expression of complement regulatory proteins could improve survival of xenogeneic islets. Transgenic porcine islets engineered to express the human complement inhibitor DAF were bound significantly less by C3 cleaved protein than control porcine islet cells and showed partial protection from MAC lysis when exposed to human serum [16]. Likewise, when porcine islet cells were engineered to express the human complement regulatory proteins CD59 and MCP, they survived in vitro when exposed to human serum [16]. Moreover, neonatal pig islets engineered with decreased expression of α-Gal and induced expression of human MAP, DAF, and CD59 complement inhibitor proteins led to reduced levels of humoral-mediated hyper acute rejection when transplanted into diabetic baboons. Still, even with these transgenic modifications, these xenografts were eventually rejected [16]. Sertoli cells (SCs) are located in mammalian testicles where they function to nourish maturing spermatogonia and protect these developing germ cells from destruction by the immune system. Protection of the auto-antigenic germ cells 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 both NPSCs and PAECs. Yet, the NPSCs were negative for the alternative pathway protein factor B, while PAECs showed variable deposition quantities. C3 deposition also occurred on both NPSCs and PAECs. Lastly, whereas PAECs showed MAC insertion, NPSCs were mostly negative for the deposition of MAC [19-21]. This implies that NPSCs are inhibiting MAC formation and/or deposition 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* cytotoxicity 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. Non-transduced NPSCs were used as controls. Exposure to the MCP or DAF short hairpin RNA (shRNA) led to a decrease in NPSC survival, to 3.7% and 9.8%, respectively, as compared to controls indicating the expression of these factors by NPSC is necessary for their inhibition of complement mediated cell lysis and survival (Figure 2). These results imply that NPSCs survive xenotransplantation through active inhibition of the complement cascade by increased expression of complement inhibitor proteins.

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.

Acknowledgement

This work was supported in part by The Jasper L. and Jack Denton Wilson Foundation (to JMD and GK), South Plains Foundation (to GK and JMD), Wright Family ASCO Foundation Endowed Presidential Scholarship (to RLW) and the SALUTE Gold Tier Scholarship (to RLW).

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