Sertoli cell expression of complement regulatory proteins is necessary to their survival of hyperacute rejection

Rachel L. Washburn[1,2], Gurvinder Kaur[2,3], Jannette M. Dufour[1,2,3]

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

BACKGROUND

- Transplantation is an important, life-saving procedure
- There is currently a shortage of transplantable tissue due to number of donors, time of tissue availability, and organ condition (Fig. 1)

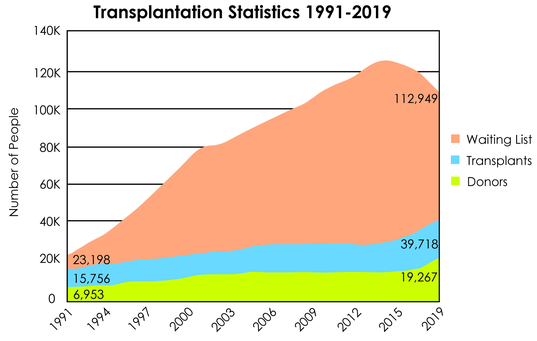

Figure 1. *Transplantation statistics from 1991 through 2019. The number of organ donors has increased by over 10,000 from 1991 to 2017. During this time, the number of people receiving transplants has increased by over 20,000. However, the number of people awaiting organ and/or tissue transplants has also increased by roughly 90,000 people. The need for transplantation procedures continues to climb at a significantly higher rate than the availability of donor organs, leading to a shortage of critically needed transplant tissue. 20 people die each day awaiting a transplant.* (Modified from www.organdonor.gov/statistics-stories/statistics.)

- Xenotransplantation, like transplanting pig tissue into humans, offers an unlimited supply of tissue and organs
- Pig tissue can be rejected by the immune system, predominantly through mechanisms of hyperacute rejection
- Hyperacute rejection is primarily mediated by antibody and spontaneous activation of the complement system (Fig. 2)

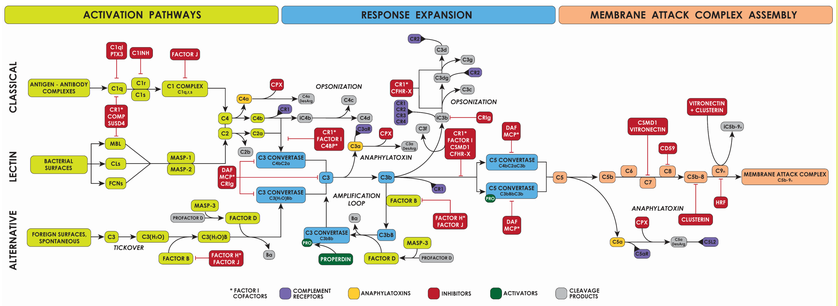

Figure 2. *Flowchart of the complement system including cascade proteins, anaphylatoxins, inhibitors, activators, cleavage products, and receptors. Complement regulatory proteins include inhibitors and activators. MBL: Mannose binding lectin. CLs: Collectins. FCNs: Ficolins. MASP: Mannose binding protein associated serine protease. C1qI: C1q inhibitor. C1INH: C1 inhibitor, SERPING1. COMP: Cartilage oligomeric matrix protein. SUSD4: Sushi-domain containing protein 4. PTX3: Pentraxin-related protein 3. DAF: Decay accelerating factor, CD55. MCP: Membrane cofactor protein, CD46. CPX: Carboxypeptidase. CRIg: Complement receptor of immunoglobulin. C4BP: C4 binding protein. PRO: Properdin. CFHR-X: Complement factor H-related protein 1-5. HRF: Homologous restriction factor, C8 binding protein. CSMD1: CUB and sushi domains protein 1. CR1: Complement receptor 1, CD35. CR2: Complement receptor 2, CD21. CR3: Complement receptor 3, CD11b/CD18. CR4: Complement receptor 4, CD11b/CD18. C3aR: C3a receptor. C5aR: C5a receptor 1. C5L2: C5a receptor 2.*

- Transplant recipients are required to undergo immunosuppressive therapies, which lead to harsh side effects such organ damage, reduced wound healing, chronic infection, and even rejection of the transplant
- Sertoli cells (SCs), an immune privileged cell type, survive xenotransplantation long-term (90+ days) without using immunosuppression (Fig. 3)

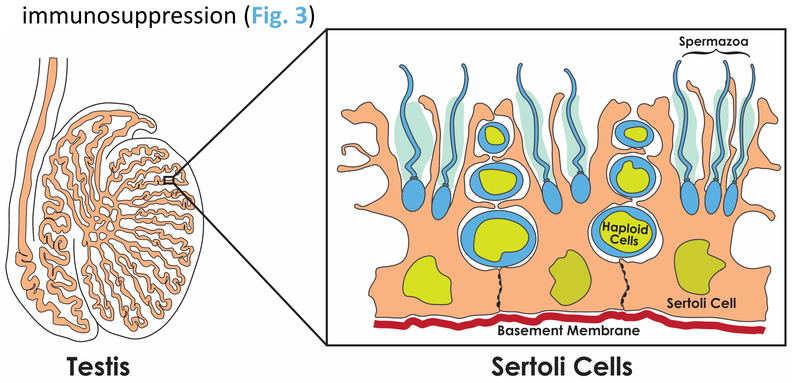

Figure 3. *SCs are an immune privileged cell found in mammalian testes that protects and nourishes maturing spermatogonia. SCs provide a physical barrier between the sperm and blood vessels, the blood testes barrier, and also confer an immune privileged environment. These ensure that sperm are protected from autoimmune destruction by the male immune system.*

OBJECTIVE

- To better understand the mechanisms of how SC grafts create an immune privileged environment to improve transplant procedures and viability

HYPOTHESIS

- A critical component to SC xenograft survival is inhibition of the complement system at multiple points through increased expression of complement regulatory proteins

METHODS

- Collect and culture neonatal pig Sertoli cells (NPSCs), islets (NPIs), and aortic endothelial cells (PAECs) from neonatal Landrace-Yorkshire pigs
- Utilize cultured cells in *in vitro* Human Serum + complement assay (Fig. 4)
- Use qPCR and Western blot to quantify mRNA and protein expression levels of complement regulatory proteins (CRPs)

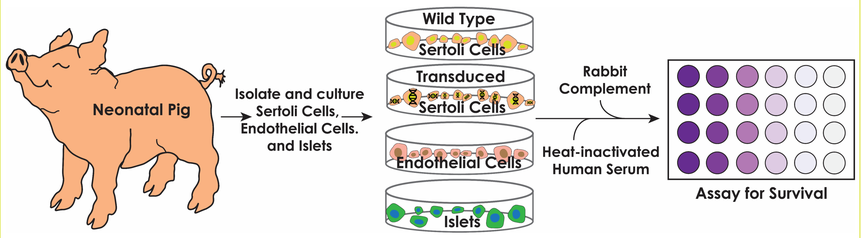

Figure 4. *Collect testicular, pancreatic, and aortic tissue from neonatal pigs. Isolate SCs, islets, and endothelial cells for culture. Knockdown expression of complement inhibitory proteins (CIPs) using shRNA. Use cultured cells in Human Serum + Complement cytotoxicity assay, and assay for survival after exposure to activated complement.*

RESULTS

- SCs survive antibody and complement exposure at significantly higher levels than endothelial and islet cells (Fig. 5)

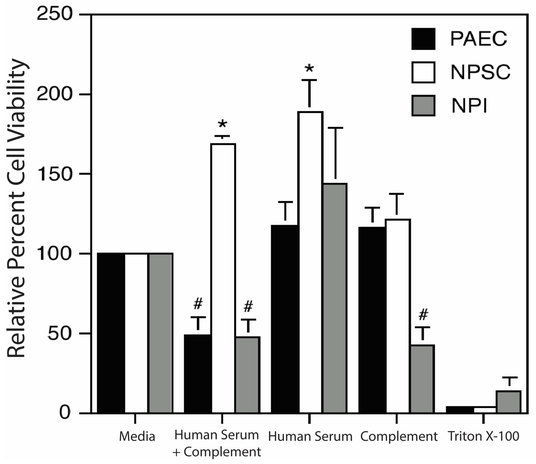

Figure 5. *Susceptibility of PAECs, NPSCs, and NPIs to human antibody/complement-mediated lysis. PAECs, NPSCs, and NPIs were exposed to media alone, human serum plus complement, human serum alone, complement alone, or Triton X-100, and viability was assessed by the MTT assay. Viability of cells exposed to media alone was normalized to 100%, and the relative percent cell viability (PAECs: black bar, NPSCs: white bar, NPIs: gray bar) for each condition was graphed. Viability is presented as the mean ± SEM for at least three different experiments performed in duplicate. NPSCs showed significantly higher viability than PAECs and NPIs when exposed to human serum plus complement, indicating NPSCs survive antibody-activated complement cascade killing. Asterisks denote a significant difference from the control values. The significance was determined by ANOVA and Fisher's PLSD with *$p \leq 0.05$; **$p \leq 0.004$; and ***$p \leq 0.0001$ (Wright et. al., 2016).*

- While complement fragments C3 and C4 are deposited on both cultured NPSCs (survive complement) and PAECs (are killed by complement), Factor B and MAC are not seen deposited on NPSCs (Fig. 6)

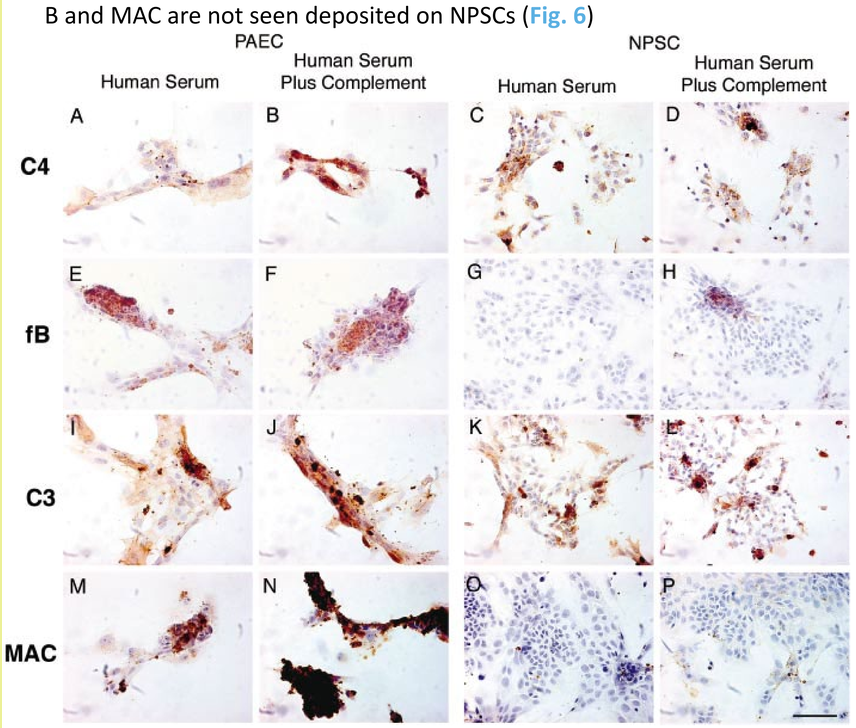

Figure 6. *NPSCs and PAECs were grown on culture slides, then exposed to the Human Serum + Complement assay with either AB human serum containing complement or human serum with rabbit complement to determine deposition of complement fragments when the complement cascade was activated by any of the three pathways of activation. PAECs were killed by complement, while NPSCs survived complement. Immunohistochemical analyses was performed of complement fragments C4 (classical and lectin pathways), Factor B (fB, alternative pathway), C3 (convergence point of the three activation pathways), and MAC (membrane attack complex, final step in complement activation). C3 and C4 are seen deposited on NPSCs and PAECs. Factor B deposition was not seen on NPSCs, but was observed on PAECs. MAC deposition was not seen on NPSCs, while PAECs were covered in MAC. These results indicate that NPSCs are inhibiting the complement cascade before MAC deposition, and possibly before amplification loop generation of the alternative pathway. This deposition needs to be quantified (Dufour et. al., 2005).*

RESULTS CONTINUED

- NPSCs have been shown to express elevated mRNA levels of the CRPs clusterin, MCP, DAF, and CD59; NPSCs also express elevated protein levels of DAF and MCP (Fig. 7a)

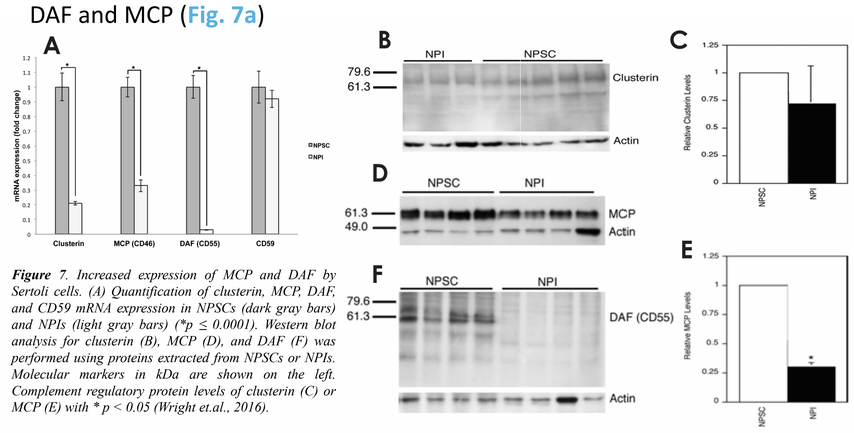

Figure 7. *Increased expression of MCP and DAF by Sertoli cells. (A) Quantification of clusterin, MCP, DAF, and CD59 mRNA expression in NPSCs (dark gray bars) and NPIs (light gray bars) (*$p \leq 0.0001$). Western blot analysis for clusterin (B), MCP (D), and DAF (F) was performed using proteins extracted from NPSCs or NPIs. Molecular markers in kDa are shown on the left. Complement regulatory protein levels of clusterin (C) or MCP (E) with * $p < 0.05$ (Wright et.al., 2016).*

- Our preliminary data indicates that MCP and DAF are crucial to SC xenograft survival of the Human Serum + Complement cytotoxicity assay *in vitro* (Fig. 8)

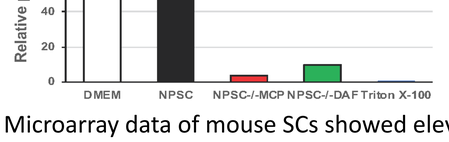

Figure 8. *Complement mediated cell lysis. To determine the role of MCP or DAF in complement mediated cell lysis of NPSC, NPSC (n=1) were transduced with MCP or DAF shRNA at 1MOI. After 24hrs, these cells were exposed to human serum plus complement and cell viability was assessed by MTT assay. Non-transduced NPSC 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 NPSC transduced with MCP (green bar) or DAF (red bar) shRNA was graphed. NPSC cultured in triton-X 100 were used as positive controls for cell lysis (Washburn et. al., 2020).*

- Microarray data of mouse SCs showed elevated expression of multiple other CRPs (Table 1)

REGULATOR	CELLULAR DISTRIBUTION	REGULATION TARGET
C1 INH	Plasma protein	C1 complex
C1qI	Plasma protein	C1 complex
CR1*	Blood cells, endothelia, neurons	C1 complex, C3 and C5 convertases
Factor J	Plasma protein	C1 complex, C3 convertase
Properdin	Plasma protein	C3 convertase
CRIg	Macrophages; gland, adipose, and intestine	C3 convertase, opsonization proteins
Factor I	Plasma protein	C3 and C5 convertases
C4BP*	Plasma protein	C3 convertase
CSMD1	Brain, testes, GI tract, placenta, thyroid	C3 convertase; MAC
Factor H*	Plasma protein	C3 convertase
CFHR-X	Plasma proteins	C3 and C5 convertases, opsonization proteins
MCP*	Most cell types	C3 and C5 convertases
DAF	Most cell types	C3 and C5 convertases
Clusterin	All tissues and all body fluids	MAC
Vitronectin	Plasma protein	MAC
CD59	Endothelia and circulating cells	MAC
HRF	Blood cells, endothelia, epithelia	MAC
CPX	Plasma proteins	Anaphylatoxins

Table 1. *Known regulatory proteins of the complement system. Regulators colored in blue have been shown by microarray analyses to have elevated mRNA expression in mouse SCs. This data gives us a starting place that we can use in determining CRP expression in NPSCs. *Cofactors of Factor I, can also act independently of Factor I.*

CONCLUSIONS

- NPSCs survive long-term as xenografts without immunosuppressive drugs
- NPSCs survive hyperacute rejection by inhibiting the complement cascade before MAC deposition
- NPSCs express the CRPs DAF and MCP at elevated levels, and when these CRPs are knocked down, NPSC survival of complement is severely diminished
- Mouse SCs express many other CRPs, which may be evolutionarily conserved
- Data obtained from these experiments will be critical in determining the mechanism behind SC immune privilege
- This could increase transplant viability clinically for patients and possibly allow for clinical use of xenografts, thus addressing the issue of organ shortage

SELECTED REFERENCES

- Washburn RL, Kaur G, Dufour JM. Sertoli cells and complement inhibitors: A possible mechanism to increase pancreatic islet viability. Ann of Diabetes Res. 2020; 4: 1013.
- Wright K, Dziuk R, Mital P, Kaur G, Dufour JM. Xenotransplanted Pig Sertoli Cells Inhibit Both the Alternative and Classical Pathways of Complement-Mediated Cell Lysis While Pig Islets Are Killed. Cell transplantation. 2016;25(11):2027-40.
- Kaur G, Thompson LA, Dufour J. Therapeutic potential of immune privileged Sertoli cells. Animal Reproduction. 2015;12:105-17.
- Dufour JM, Hamilton M, Rajotte RV, Korbutt GS. Neonatal porcine Sertoli cells inhibit human natural antibody-mediated lysis. Biology of reproduction. 2005;72(5):1224-31.
- Mital P, Kaur G, Dufour JM. Immunoprotective sertoli cells: making allogeneic and xenogeneic transplantation feasible. Reproduction. 2010;139(3):495-504.

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