XENOTRANSPLANTATION –
Making Pigs Fly

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Waitlist and Transplant Activity for Kidneys, 2000-2009

Source: OPTN/SRTR Annual Report Tables 1.3, 1.6, 1.7
Mismatch Between Islet Donors and Recipients

150,000

- Type 1 Diabetes: 160,000
- Organ Donors: 45
- Pts Transplanted: 190
There are not enough donors
Why Pig as a Donor

- Few Ethical Problems
- Potential for genetic manipulation
- Domesticated for thousands of years
- Reaches sexual maturity at an early age
- Breeds easily, multiple progeny
- Physiologically similar to humans
HAR in Xenotransplantation

- Preformed anti-Pig Antibodies
- Complement Activation
- Thrombosis
Preformed Anti-Pig Antibodies in Humans

• Both IgG and IgM preformed antibodies detected
• > 95% anti-bodies directed against the carbohydrate epitope: Galactose $\alpha 1,3$ Galactose
• GAL Transferase gene found as a pseudogene in Humans and Old World Monkeys
• Galactose $\alpha 1,3$ Galactose carbohydrate not expressed in Humans or Old World Monkeys
ENDOTHELIAL CELL SURFACE CARBOHYDRATES

- PIG
  - βGal1,4 β GlcNac-R Lactobiose
  - α Gal1,3Gal α1,3 gal transferase

- HUMAN
  - α1,2 Fuc H transferase
  - A: GalNAc α1,3 A transferase
  - B: Gal α1,3 B transferase
XENOGRAFTS Lead to COMPLEMENT Activation

**Normal Endothelium**
- Anti pig IgM binds to endothelium
- C1 activates C2 and C4
- C4b and C2a form the C3 convertase
- The addition of C3b forms the C5 convertase
- Subsequent formation of the MAC

**Transgenic Endothelium**
- Anti pig IgM binds to endothelium
- RCA binds to endothelium
- C1 activates C2 and C4
- DAF splits the C3 convertase
- MCP inactivates the C5 convertase
- CD59 blocks the formation of the MAC
Hyperacute Rejection in Xenotransplantation
Disordered Thromboregulation

\[ \text{loss of ADPase, AT-III and TM} \]

Macrophage and NK Cell Recruitment and Activation

Cytokines, tissue factor, lectins

Type II EC Activation

Leukocyte adhesion, procoagulant response, cytokines (IL-8, MCP-1 etc.)
Issues in Xenograft Rejection

• Xenograft Rejection due to Receptor Ligand incompatibilities between Donor and Recipient
• Leads to activation of Innate Immune System
  – Preformed antibody, Complement, Thrombosis, NK cells, Macrophages
• Successful Allotransplantation is achieved by selective manipulation of cognate immune system
• We do not have the capacity to suppress the innate immune system
Coagulation: initiation phase

injured vessel

TF

VII → VIIa

VIIa/TF

IX → IXa

X → Xa

prothrombin → thrombin

fibrinogen → fibrin
Coagulation: Propagation Phase

- VII → VIIa
- VIIa/TF
- IX → IXa
- XI → XIa
- VIII → VIIIa
- X → Xa
- prothrombin
- thrombin
- fibrinogen
- fibrin
- platelet activation

injured vessel

TF

Activated platelet

RBC
Initiation: Regulation by TFPI

injured vessel

TF

VIIa

VIIa/TF

IX

Xa

IX

IXa

X

Xa

prothrombin

thrombin

endothelial cell surface

fibrinogen

fibrin

TFPIα • K-1, K-2 & K-3
  • bound to a GPI-linked co-receptor and associated with GAGs

TFPIβ • K-1 & K-2 only
  • directly GPI-linked
Injured vessel

Propagation: regulation by thrombomodulin

- Thrombomodulin (transmembrane-anchored)
- Thrombin
- Platelet activation

Factors and Processes:
- Prothrombin
- Fibrinogen
- Fibrin
- Platelet activation

Enzymes and Proteins:
- VIIa
- VIIa/TF
- IX
- IXa
- X
- Xa
- Va
- V
- VIII
- VIIIa
- XI
- XIa

Diagramatic Representation:
The role of the protein C pathway in regulating coagulation

CD39/ ATPDase – Can prevent platelet activation by Thrombin

- Platelet recruitment
- Platelet adhesion
- Platelet activation
Unmodified Islet Xenografts Survive > 6 Mo following Cellular Immune Suppression

Group A = basiliximab, everolimus and FTY720
Group B = basiliximab, everolimus and FTY720 + anti-CD154mAb
Group C = basiliximab, everolimus and FTY720 + anti-CD154 mAb + leflunomide

Hering et al. Nat. Med. 2006, 12; 301.
Islet xenograft function with hCD46 Tg Pig islets

Immunosuppressive Protocol

Van der Windt et al. AJT 2009, 9; 2716
Islet xenograft function with hCD46 Tg Pig islets

Control Islets

hCD46 Tg Islets

BSL + Insulin
C-peptide

BSL + Insulin
C-peptide

Van der Windt et al. AJT 2009, 9; 2716
Xenotransplantation
- Current Understanding

• Transgenic expression of Complement Regulators or CD39 or Deletion of αGal independently prevent HAR
• Combined genetic manipulations essential for prolonged survival
• Prolonged survival of Islets without Genetic Manipulation achieved in primates
• Use of Genetic Modification likely to prolong Islet survival substantially
• Suppression of the Cell mediated response likely to be a significant hurdle
Strategy to prevent IBMIR and preserve islet mass post transplantation

Preformed AntiGal Ab  
Deletion of Gal (α Gal -/-)

Complement activation  
Complement regulatory proteins CD55

Tissue Factor/Thrombosis  
Expression of human TFPI and TM on pig islets

Platelet activation  
Expression CD39 on pig islets

T cell mediated response  
Local Immune suppression
ZOONOSES

- Infection by organisms pathogenic to humans and pigs
- Infection by unique porcine pathogens with potential for opportunistic infection in immunocompromised host
- Porcine specific viruses & pathogens infecting the transplanted organ & avoiding immune surveillance by host immune system
- Porcine endogenous retroviruses recombining human endogenous retrovirus creating emerging new infection & risk to community